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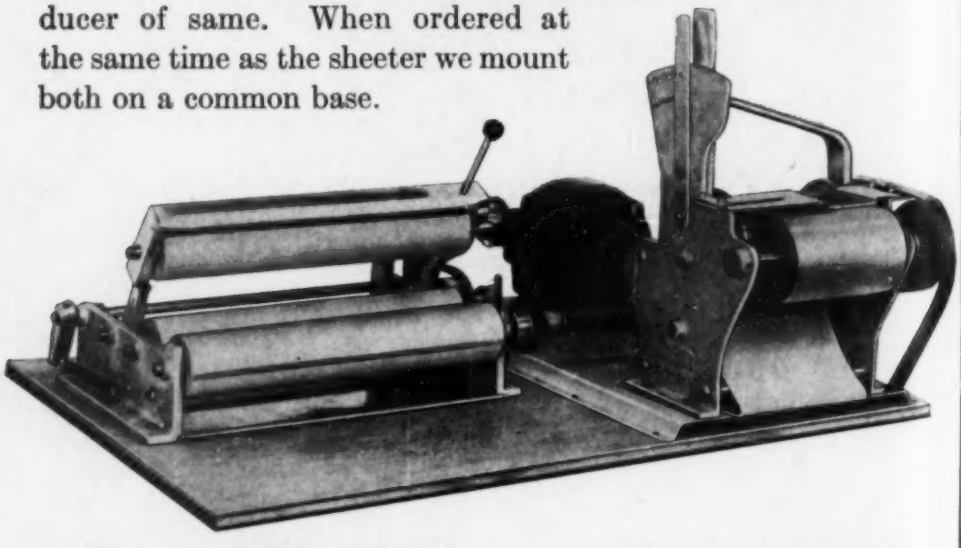

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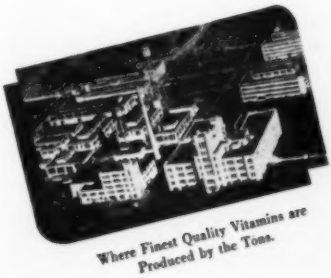

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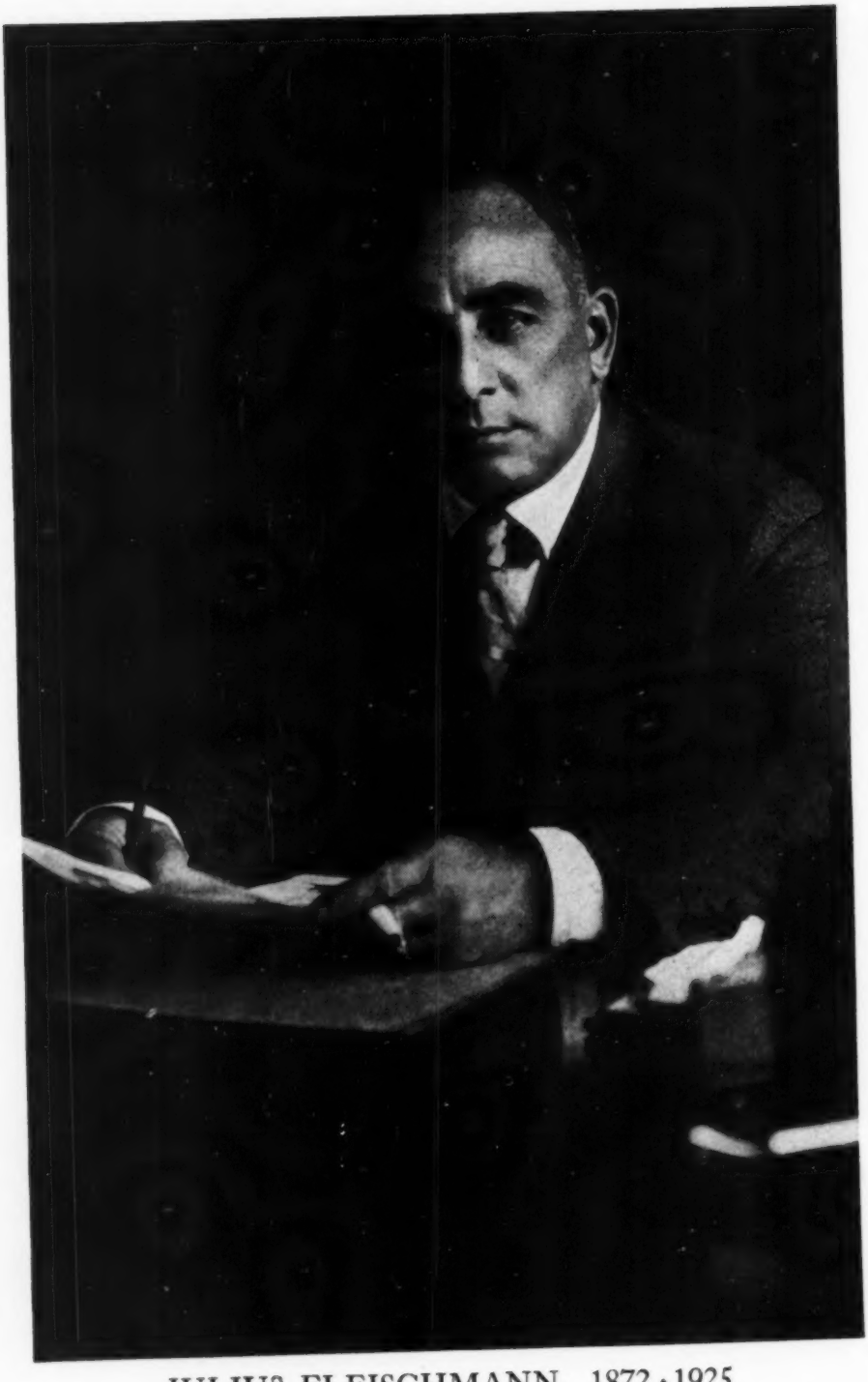

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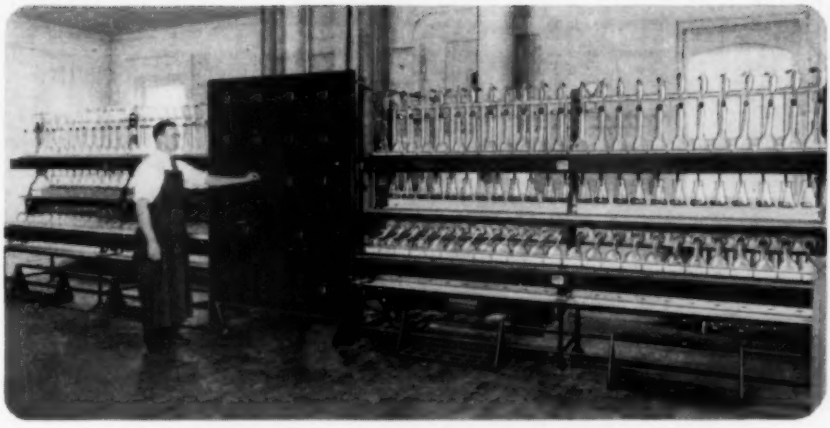

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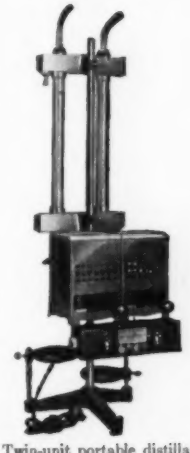

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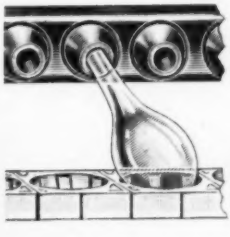

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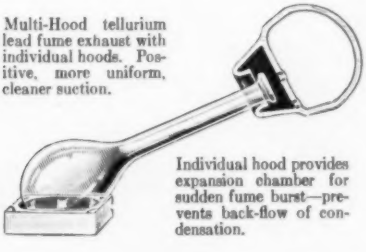

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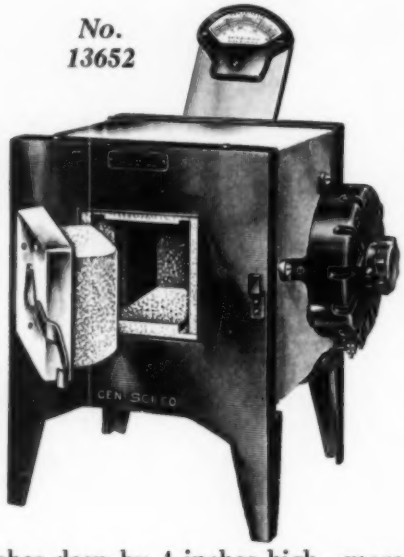

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CEREAL CHEMISTRY

VOL. XX NOVEMBER, 1943 NO. 6

THE NUTRITIVE VALUE OF WHITE AND WHOLE WHEAT BREADS

ROBERT F. LIGHT and CHARLES N. FREY

The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

(Read at the Annual Meeting, May 1942; manuscript received for publication June 21, 1943)

During the present crisis in the world's food supply, bread and other wheaten products must assume a more important role in the nutrition of man. Consequently, studies which may contribute to our knowledge of the nutritive value of bread are particularly pertinent.

The development of the "enriched bread" program is an important step in the attempt to improve the national health, and our findings cannot detract from the value of enriched bread in fulfilling the function for which it was designed, namely, restoring to bread some of the nutritive factors which have been removed by the modern milling process. However, if during an emergency bread should constitute the major portion of our food intake, any possible deficiencies should be well known and fully appreciated so that they may be corrected or adequately compensated for by the balance of the diet.

The nutritive value of bread will be limited by the biological value of the protein of bread. Chick (1942) has determined the biological value of white, whole meal, and "National Wheat Meal" (85% extraction) flours. She found that the proteins of whole meal and National Wheat Meal have advantages of 17–24% and 13–16%, respectively, over those of white flour. Jones and Devine (1942) report that soybean, peanut, and cottonseed flours, when mixed at 5, 10, or 15% levels with white flour, produce products with markedly improved proteins. The supplementary value of these flours is attributed to their lysine content. Mitchell *et al* (1943) state that enriched white bread with 6% skim milk solids and whole wheat bread have about equal value in the promotion of growth, but that a combination of these two supplements will be better than either one alone. They did not determine whether doubling of either supplement would produce

an effect equal to that of combining the two supplements. Fairbanks (1938) demonstrated by ad libitum feeding that 12% milk bread is superior in growth-promoting properties to a 6% milk bread. By the paired-feeding technique, Fairbanks (1939) did not obtain significantly better growth with 12% milk bread as compared with 6% milk bread when the food intake was equalized. Frey *et al* (1940) have enumerated the contributions of milk to the nutritive value of bread and pointed out that bread needs additional lysine and valine.[1]

The concentrations of vitamins and minerals in bread will also affect its nutritive value. Sherman and Pearson (1942) report the results of feeding white rats with 6% milk bread, alone and supplemented variously with butter, whole milk, meat, and peas. Animals fed 6% milk bread alone died after about 100 days, presumably from a vitamin-A deficiency. Animals fed the same bread with 10% butter survived and grew reasonably well. Chick (1940) determined that white flour is deficient in one or more of the G-complex factors and states that preliminary studies indicate that the deficiency of riboflavin is the most serious. She found that whole meal has an adequate supply of the G-complex factors to support good growth during the 4-week test period. Mitchell *et al* (1943) found that a 6% skim milk bread is superior in growth-promoting properties to an enriched bread supplemented with calcium and riboflavin. We may interpret this observation as indicating that an important nutritional deficiency of white bread from the viewpoint of growth promotion is in the protein fraction.

Henry and his coworkers (1941) have studied the effect of additions of dried skim milk and dried whey on the nutritive value of white bread. From their data they conclude that the marked beneficial effects of milk or whey were the result of an increase in the calcium of the bread and to a large extent to the increase in riboflavin and other members of the "B_2-complex." The results certainly indicate that their control breads were critically low in calcium (0.027% to 0.03% calcium on the dry basis). Their second experiment using bread as an exclusive diet, when considered with the data of their third experiment, indicates that calcium was one of the major deficiencies of their control bread. However, we would interpret the results obtained in their third experiment as indicating that when the calcium deficiency is corrected, the next most important contribution of milk to the nutritive value of bread is its protein. Our interpretation of their data appears to us as tenable as the conclusions offered by the authors, i.e., that the primary contribution of milk to white bread, aside from calcium, is riboflavin and other members of the "B_2-complex."

[1] This was erroneously quoted as tryptophane in the published article.

In the present investigation, bread, fed ad libitum, was made the sole food for white rats except for certain essential nutritive factors which are not present in bread in adequate amounts, yet are necessary for the growth of these test animals. By appropriate supplements it was possible to demonstrate that the breads studied were deficient in certain specific essential nutrients, but that other dietary essentials were present in adequate amounts.

Experimental

Procedure. The index of the nutritive value of the breads was the growth of young weaned white rats, weighing 50 to 60 g at the start of the experiments. They were fed air-dried breads exclusively, except for a daily supplement of four drops of a solution of fat-soluble vitamins in corn oil which supplied 80 units of vitamin A, 20 units of D, and 250 μg alpha-tocopherol. The breads were made up about once a week, using the same basic formula, Table I, which was modified

TABLE I

BASIC BREAD FORMULA

Ingredient	Parts
Flour	100.0
Water	64.0
Yeast	2.0
Sugar	5.0
Salt	2.0
Shortening	3.5
Diamalt, dry	1.0
Dough conditioner	0.33

only by the addition of certain supplements, in accordance with the plan of the experiments.

The white breads were made with a Northwestern patent flour, (protein 13.5%, ash 0.44 to 0.46%), while the whole wheat breads were made with 100% whole wheat flour. The white bread (air dried)[2] had an average protein content of 12.48% and the whole wheat bread, 14.25%. The dry yeast used contained 50% protein, and the dry skim milk, 35% protein. The "dough conditioner" included in our basic bread formula contributes calcium to the dough, and air-dried bread made according to this basic formula contains approximately 0.05% calcium.

When the basic formula was modified by the inclusion of dry skim milk, salts, or casein, these materials were added as 2, 4, or 6% based on the flour in the basic formula.

[2] This bread was air dried in the dark.

The amino acids are given as the per cent of these compounds present in the dried bread fed to the animals. The amino acids [3] used in these studies were dl-lysine.HCl, l(+)-valine, dl-leucine, l(−)-tryptophane, dl-methionine, l(+)-histidine, and l(+)-isoleucine. The calculated amount required by the formula was added to the flour in making the bread.

In this series of experiments all of the animals in any one experiment were comparable. However, animals from three sources (and strains) were used during this study; consequently, we shall restrict the comparison of results to the groups within a single experiment. Each group consisted of 10 animals, except those fed breads containing amino acids. These groups contained five animals each.

The use of white rats as test animals has certain inherent limitations, owing to definite differences in the dietary requirements of the rat compared with man. The requirements of white rats for a dietary source of niacin are so low that our study gives little information on bread as a source of niacin for humans. Likewise, rats require no dietary source of vitamin C. However, bread is not regarded as a satisfactory carrier of this vitamin.

The white bread used in this study averaged about 120 International units B_1 per pound loaf, or 110 μg/100g of dry bread. This thiamine level is adequate for excellent growth of white rats when they are fed a bread properly supplemented with other required nutritive factors. White bread is not considered to be a source of the fat-soluble vitamins, consequently vitamins A, D, and E were given as daily supplements to all the animals fed breads.

Results

Protein deficiencies. The data obtained from the experiment reported in Table II indicate the importance of correcting the protein deficiencies of bread to improve its nutritive value.

The animals fed a basic-formula white bread or an "enriched" bread without milk made very poor gains owing to specific deficiencies of amino acids, vitamins, and, possibly, minerals. The fully enriched bread (Group 3) with added calcium and riboflavin gave better growth than the white bread or bread with added thiamine, iron, and niacin ($P = 0.01$ and 0.05 respectively). This effect may possibly be due to the added calcium, in view of the results reported by Henry and his coworkers (1941).

The animals fed a 6% milk bread grew slightly better than those on the whole wheat bread, although the difference is not significant ($P = 0.2$). The milk raised the protein content of the bread 2%, yet

[3] Obtained from the Amino Acid Manufacturers, University of California, Los Angeles, Calif.

the addition of 0.67% dl-lysine.HCl and 0.267% l(+)-valine to bread produced a significantly better rate of growth than did the 6% milk bread ($P = .01$). Ratner *et al* (1943) have shown that when d(−)-lysine is fed, 50% is excreted as such in the urine, 19% of the α amino nitrogen is excreted as urea and ammonia, and 21% is found in the

TABLE II

WEIGHT GAINS AND FOOD CONSUMPTION OF RATS FED VARIOUS BREADS

Group	Diet	First 8 weeks of test period				12-week test period
		Ave. gain	Standard deviation of mean	Ave. total food consumption	Ave. gain per gram food intake	Ave. gain
		g	*g*	*g*	*mg*	*g*
1	White bread	40	2.63	419	89	66
2	White bread + HiB_1 yeast, 15.9 mg $FePO_4.4H_2O$, 4.0 mg niacin[1]	42	3.16	439	95	70
3	White bread + 1 mg B_1, 4.0 mg niacin, 15.9 mg $FePO_4.4H_2O$, 1.288 gm $CaSO_4$, 0.8 mg riboflavin[1]	50	2.3	462	113	85
4	Whole wheat bread	75	2.6	517	145	110
5	White bread + HiB_1 yeast, 6% dry skim milk solids, 4.0 mg niacin, 15.9 mg $FePO_4.4H_2O$[1]	81	3.3	501	161	121
6	White bread + HiB_1 yeast, 0.67+ dl-lysine.HCl, 0.267% l(+)-valine, 2.0% O and M salts[2], 0.8 mg riboflavin[1]	109	6.9	518	211	160
7	Whole wheat bread + 6% dry skim milk solids	111	4.3	514	216	163
8	White bread + HiB_1 yeast, 0.938% dl-lysine.HCl, 0.405% l(+)-valine, 2.0% O and M salts, 0.8 mg riboflavin[1]	123	6.4	500	246	168
9	Dog chow	171	6.8	786	218	219

[1] Per loaf. Each loaf was made according to the formula in Table I, using 300 g of flour and other ingredients in proportion. The air-dried loaves weigh approximately 320 g. Each loaf made with HiB_1 yeast (2%) contained at least 450 International units per loaf.
[2] Osborne and Mendel (1919) salt mixture.

various amino acids of the body tissues. Better growth was obtained by the addition of 0.755% amino acids (lysine and valine) than by the addition of 2% protein (supplied by the 6% milk). Of this 0.755% amino acid supplement about one third or 0.244% was in the form of d(−)-lysine, and only 21% of the α amino nitrogen of this compound

is utilized in building tissue protein. The supplementary effect of lysine and valine appears to be that of supplying specific amino acids which are not adequately supplied by the bread protein, and not merely an effect of raising the nitrogen content of the bread.[4]

Increasing the lysine and valine supplement to the bread from 0.67% dl-lysine. HCl and 0.267% l(+)-valine to 0.938% and 0.405% respectively did produce better growth, but this difference was not statistically significant ($P = 0.2$).

The average food consumption of the individuals in groups 4, 5, 6, 7, and 8 were all between 500 and 518 g for the first 8 weeks of the test period. Yet the average gains ranged from 75 to 123 g and the

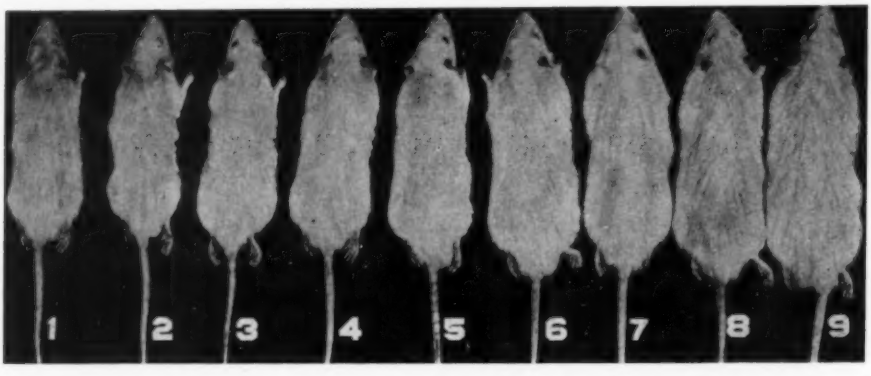

Fig. 1. Representative animals from Groups 1 through 9 tabulated in Table II. (Photograph taken on 87th day of test).

Group 1—White bread
Group 2—Enriched white bread
Group 3—Completely enriched white bread
Group 4—Whole wheat bread
Group 5—White bread with milk, niacin, and iron
Group 6—White bread with lysine, valine, salts, and riboflavin
Group 7—Whole wheat bread with milk
Group 8—White bread with lysine, valine, salts, and riboflavin
Group 9—Dog chow

"nutritive efficiency" of the breads (mg weight gain per g of food consumed) varied from 145 to 246. Data in Table XII supports the view that the improved nutritive value is correlated with increased lysine and valine content of the breads. The addition of lysine and valine to white bread more than doubled the calculated "nutritive efficiency" of the bread, increasing this value from 90–100 to more than 200 for the 8-week period.

The use of 6% dry skim milk solids in the production of white bread improves the nutritive value of bread by (1) partially correcting

[4] We have found that the addition of 0.6% glycine to a white bread adequate in minerals and vitamins had no effect on its growth promoting value for white rats, whereas the addition of 0.33% l(+)-lysine and 0.27% l(+)-valine (total 0.6%) resulted in gains equal to three times that of the control white bread or the 0.6% glycine bread (15 g/week vs. 5 g/week over a six-week test period).

the amino-acid deficiency of white bread, (2) increasing the riboflavin content of the bread, and (3) improving the mineral content.

The value of dry skim milk solids for improving the nutritive value of white or whole wheat bread will be quite evident from an examination of Figure 1. A comparison of the size of a representative animal fed "enriched" bread without milk (No. 2) with that of animals fed 6% milk bread (No. 5) or a bread with added amino acids, salts, and riboflavin (No. 8), emphasizes the contribution milk can make to the nutritive value of bread. This supplementary value of milk is also indicated by a comparison of the animals fed whole wheat bread (No. 4) and whole wheat 6% milk bread (No. 7).

Further evidence that 6% milk does not completely correct the protein deficiencies of white bread is presented in Table III. The

TABLE III

WEIGHT GAINS OF RATS SHOWING EFFECT OF ADDED PROTEIN ON NUTRITIVE VALUE OF 6% MILK BREAD

Diet	Average gain	
	8 weeks	12 weeks
	g	g
White bread + 6.0% dry skim milk 2.0% O and M salts	50.8	78.7
White bread + 6.0% dry skim milk 2.0% O and M salts 0.8 mg riboflavin per loaf	47.8	75.3
White bread + 6.0% dry skim milk 2.0% casein 2.0% O and M salts 0.8 mg riboflavin per loaf	83.5	124.8

addition of riboflavin had no effect on the growth-promoting properties of a 6% milk bread, whereas the addition of 2% casein (or, in effect, doubling the protein supplement) effected a marked enhancement of the growth-promoting value of the bread. This confirms the results of Fairbanks (1938) indicating that 12% milk bread was better than 6% milk bread.

None of the groups fed the supplemented breads equaled the gains made by the group fed dog chow which contained 22% protein.

In an experiment designed to investigate amino-acid deficiencies, it was found (Table IV) that white bread is deficient in both lysine and valine.

Lysine is apparently of more significance in limiting the nutritive value of basic white bread than valine. However, both are involved. We have found also that 2% vitamin-free casein does not fully supple-

TABLE IV

WEIGHT GAINS OF RATS SHOWING EFFECTS OF AMINO ACIDS ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks	
	Average	Standard deviation of mean
	g	g
White bread + 0.67% dl-lysine.HCl 3.0% O and M salts 20γ riboflavin per day	70	3.18
White bread + 0.267% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	37	3.08
White bread + 0.67% dl-lysine.HCl 0.267% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	98	3.54
White bread + 0.36% dl-lysine.HCl 0.16% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	66	3.81

ment the protein of white bread, although 4% casein permits as good growth as the approximate equivalents of lysine and valine supplements to white bread.

The possibility that other essential amino acids might be present

TABLE V

WEIGHT GAINS OF RATS SHOWING EFFECT OF AMINO ACIDS ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks		Food intake	Gain per gram food intake
	Average	Standard deviation of mean		
	g	g	g	mg
White bread + 0.67% dl-lysine.HCl 0.267% l(+)-valine 0.133% dl-leucine 0.13% l(−)-tryptophane 0.267% dl-methionine 3.0% O and M salts + 20γ riboflavin per day	95	6	509	187
White bread + 0.67% dl-lysine.HCl 0.267% l(+)-valine 3.0% O and M salts + 20γ riboflavin per day	94	3.1	573	164
Whole wheat bread	75	2.9	480	156
White bread + 2% casein 3% O and M salts + 20γ riboflavin per day	72	2.0	461	156
White bread	32	2.5	337	95

in white bread at suboptimal levels was considered. The addition of the three amino acids, leucine, methionine, and tryptophane, did not improve the growth-promoting value of a white bread already supplemented with lysine, valine, riboflavin, and salt mixture (Table V). Both of the amino-acid-supplemented breads gave significantly better growth than either whole wheat bread or the white bread made with 2% casein.

TABLE VI

WEIGHT GAINS OF RATS SHOWING EFFECT OF AMINO ACIDS ON NUTRITIVE VALUE OF BREAD

Group	Diet	First 8 weeks of test period			
		Gain		Ave. food intake	Nutritive efficiency [1]
		Average	Standard deviation of mean		
		g	g	g	
1	White bread + 0.8 mg riboflavin, 4.0 gm A, D, E oil, 4.0% O and M salts (per loaf)	47.0	2.85	494.3	95.08
2	White bread + 0.8 mg riboflavin, 4.0 gm A, D, E oil, 4.0% O and M salts, 0.8% l(+)-lysine, 0.3% l(+)-valine (per loaf)	123.0	11.5	543.0	226.0
4	White bread + 0.8 mg riboflavin, 4.0 gm A, D, E oil, 4.0% O and M salts, 0.8% l(+)-lysine, 0.3% l(+)-valine, 0.2% dl-methionine, 0.25% l(+)-histidine, 0.1% l(−)-tryptophane, 0.1% l(+)-isoleucine (per loaf)	143.0	18.5	585.2	244.3
12	Purina dog chow	198.0		917.3	215.8

[1] Mg gain per gram food eaten at 8 weeks.

The addition of 2% casein as well as salts and riboflavin produces a bread equal to whole wheat bread for the growth of rats. Since we have found that a 6% milk bread equals a whole wheat bread, confirming the results of Mitchell *et al* (1943), and we obtain the same result by adding only the vitamin-free protein equivalent of 6% milk plus riboflavin and minerals, it appears that the important contributions of milk to the nutritive value of bread are the protein, minerals, and riboflavin, and not as suggested by Henry *et al* (1941), the other members of the "B complex."

Another combination of amino acids which may be present at sub-

optimal levels in bread includes histidine, isoleucine, tryptophane, and methionine in addition to lysine and valine. When these four amino acids were added to a bread supplemented with 0.8% l(+)-lysine and 0.3% l(+)-valine plus salts and riboflavin, the gains made by the animals were better than those obtained with the lysine-valine supplemented breads (Table VI).

This average difference, however, is not significant ($P = 0.3$), but one must keep in mind that only five animals were used in each group when amino acids were added to the bread. Both amino-acid-supplemented breads had a nutritive efficiency, calculated for the 8-week period, of more than twice that of the basic-formula bread made with riboflavin, salts, and A, D, E oil and slightly above that of the dog chow, although their growth-promoting value did not equal that of the dog chow diet.

TABLE VII

WEIGHT GAINS OF RATS SHOWING EFFECT OF MILK AND YEAST ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks		Food intake	Gain per g food intake
	Average	Standard deviation of mean		
	g	*g*	*g*	*mg*
White bread	41.0	2.87	523	104
White bread + 6% skim milk dry	89.0	5.0	527	156
White bread + 5% dry yeast	112.5	5.54	584	174

Dry yeast, which can be produced rapidly and efficiently from carbohydrates and inorganic nitrogen, has been proposed as a source of protein during the present crisis in the world's food supply. This material usually contains approximately 50% protein. The inclusion of 5% dry yeast in a white bread raises the lysine content from 0.22% to 0.39%, whereas 6% dry skim milk raises the lysine to 0.34%. The growth responses of animals fed these breads were in the same order as the lysine content (Table VII) and the difference in gain is significant in each case ($P = 0.01$ for the first and second group and 0.02 for the second and third).

Dry yeast may be considered as a good source of lysine since it contains about 3.4% of this compound.

Vitamin deficiencies. The riboflavin content of basic white bread is too low to permit good growth when the amino-acid deficiencies have been partially corrected by the addition of 2% vitamin-free casein. This effect is shown in Table VIII. Neither the addition of casein nor

TABLE VIII

WEIGHT GAINS OF RATS SHOWING EFFECT OF RIBOFLAVIN AND PROTEIN ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks	
	Average	Standard deviation of mean
	g	g
White bread	29	2.9
White bread + 2.0% casein	35	5.3
White bread + 20γ riboflavin per day	25	6.0
White bread + 2.0% casein + 20γ riboflavin per day	54	9.6

of riboflavin produced a significantly better rate of gain over that given by the white bread. The combination of the two supplements, however, did permit significantly better growth.

While the growth of animals fed a white bread supplemented with mineral salts, riboflavin, and either the amino acids, lysine and valine, or 4% casein (vitamin-free) is good, it is not optimal. Aside from riboflavin, apparently none of the B-complex factors available in pure form at the time this experiment was carried out is important in limiting the growth-promoting value of this bread.

It is possible that if the protein or other unknown deficiencies of white bread were completely eliminated, we might be able to demon-

TABLE IX

WEIGHT GAINS OF RATS SHOWING EFFECT OF THE PURE VITAMINS OF THE B COMPLEX ON THE NUTRITIVE VALUE OF BREAD

Diet	Average gain	
	4 weeks	8 weeks
	g	g
White bread + 2.0% O and M salts, 4.0% casein, 4.0 g A, D, E oil[1], 300γ B_1, 500γ calcium pantothenate, 600γ riboflavin, 300γ pyridoxine, 2.5 mg niacin, 25.0 mg choline } per loaf	68	137
White bread + 2.0% O and M salts, 4.0% casein, 0.8 mg riboflavin, 4.0 g A, D, E oil } per loaf	61	131

[1] In this experiment the A, D, E oil was incorporated in the bread, and not given as a daily supplement as in previous experiments.

strate that certain of the B-complex factors are not present in optimal concentrations.

When thiamine, calcium pantothenate, pyridoxine, niacin, and choline, as well as riboflavin, were added to a 4% casein bread, they did not produce significantly better growth (Table IX). Biotin and inositol were not included in the pure vitamin supplement. However, we do not believe the bread was deficient in these factors. There may have been a deficiency of folic acid, but at the time the experiment was conducted this substance was not available.

Reproduction studies were carried out with groups identical to those given in Table IX. The pure-vitamin-supplemented animals reared 70% of the litters cast, while the unsupplemented animals

TABLE X

WEIGHT GAINS OF RATS SHOWING EFFECT OF MINERAL SALTS ON THE NUTRITIVE VALUE OF WHITE BREAD

Diet	Gain in 8 weeks	
	Average	Standard deviation of mean
	g	g
White bread + 2.0% casein 20γ riboflavin per day	75.8	2.9
White bread + 2.0% casein 3.0% O and M salts 20γ riboflavin per day	103.3	7.3
White bread + 0.67% dl-lysine.HCl 0.267% l(+)-valine 20γ riboflavin per day	86.2	6.3
White bread + 0.67% dl-lysine.HCl 0.267% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	108.0	3.2

reared only 25% of the litters cast. The dog chow group raised 90% of their litters. It appears that for reproduction the 4% casein white bread does not have an adequate supply of the B-complex factors other than riboflavin.

Mineral deficiencies. Our data on the mineral deficiencies of breads are not complete. However, as shown in Table X, we have evidence indicating that, when the protein deficiencies are improved by the addition of casein or amino acids and the riboflavin deficiency is corrected, the increased growth thus obtained causes mineral deficiencies to become apparent, which can be corrected by the addition of the Osborne and Mendel (1919) salt mixture.

These results indicate that certain salts contained in the Osborne and Mendel salt mixture are needed to permit the fullest growth rate

possible with supplemented breads. The major effect of the added minerals may possibly be attributed to the calcium in the salt mixture (cf. Henry *et al* 1941).

Discussion

The classical experiments of Osborne and Mendel (1914) demonstrated that wheat protein and particularly the constituent, gliadin, is deficient in the essential amino acid, lysine. The nutritive value of wheat or bread protein may be limited also by a relative deficiency of other essential amino acids. Rose (1937) and his coworkers have demonstrated that a suitable mixture of amino acids can serve as a nutritionally satisfactory substitute for a complete protein in the diet

TABLE XI

Essential Amino Acids in the Diet of the White Rat

Amino acids	Tentative requirements Rose (1937)	White bread (Calc.)	White bread with 6% dry skim milk (Calc.)	White bread with 2% casein (Calc.)	White bread with 4% casein (Calc.)
	%	%	%	%	%
l(+)-lysine	1.0	0.22	0.34	0.34	0.46
l(−)-tryptophane	0.2	0.10	0.13	0.13	0.15
l(−)-histidine	0.4	0.21	0.26	0.24	0.28
l(−)-phenylalanine	0.7	0.82	0.96	0.93	1.04
l(−)-leucine	0.9	1.55	1.88	1.85	2.15
l(+)-isoleucine	0.5	0.42	0.53	0.52	0.62
l(−)-threonine	0.6	0.82	0.91	0.92	1.00
l(−)-methionine	0.6	0.41	0.50	0.47	0.53
l(+)-valine	0.7	0.42	0.54	0.52	0.63
l(+)-arginine	0.2	0.42	0.50	0.52	0.62

of growing animals. He has shown that there are 10 amino acids which are "essential" and has given "tentative" minimum requirement values for the white rat.

A comparison of Rose's (1937) estimated minimum amino acid requirements of a diet with the values for these amino acids in the various white breads used in this study is given in Table XI. These calculated values are based on analyses [5] of the yeast, white flour, dry skim milk, and casein. According to these data, white bread would be considered very deficient in lysine, moderately deficient in tryptophane, histidine, and valine, and slightly deficient in methionine and isoleucine.

[5] The analyses of these materials were made by Dr. R. J. Block. Some of these results have been published in "The Determination of the Amino Acids" by R. J. Block and D. Bolling. Burgess Publishing Company, Minneapolis, Minn. 1940.

The l(+)-lysine and l(+)-valine content of the various breads used in this study are given in Table XII.

White bread, which is admittedly deficient in lysine, contains 0.22% of this amino acid. Valine is also present in amounts less than the minimum given by Rose (1937). Adding these amino acids to white bread at levels of 0.67% dl-lysine.HCl and 0.27% l(+)-valine, raises the l(+)-lysine content to 0.47% or 47% of the tentative minimum, while the l(+)-valine is raised to 0.69% or essentially equal to the tentative minimum requirements. Increasing the l(+)-lysine to 0.60% did not appreciably increase the growth rate of white rats.

The present study indicates that white bread without milk solids is not a satisfactory food for the growth of white rats. This white bread made according to our basic formula did, however, support slow

TABLE XII

L(+)-LYSINE AND L(+)-VALINE CONTENT OF BREAD STUDIED

Bread	l(+)-lysine	l(+)-valine
Rose (1937) tentative requirement	1.0	0.7
White bread	0.22	0.42
White bread + 6% dry skim milk	0.34	0.54
White bread + 2% casein	0.34	0.52
White bread + 4% casein	0.46	0.63
White bread + 0.67% dl-lysine.HCl 0.27% l(+)-valine	0.47	0.69
White bread + 0.36% dl-lysine.HCl 0.16% l(+)-valine	0.36	0.58
White bread + 0.938% dl-lysine.HCl 0.405% l(+)-valine	0.60	0.82

growth over a period of 28 weeks. The animals of Group 1, Table II, were kept for this period, and the gains amounted to about 4 grams per week. We did not observe the characteristics of calcium deficiency which Henry *et al* (1941) reported for animals fed white bread; however, our bread contained 0.05% calcium as contrasted with 0.03% in their bread, and our animals received vitamin D.

The addition of 6% dry skim milk to the formula produces a bread which is equal to whole wheat bread for growth. This observation agrees with the report of Mitchell *et al* (1943).

The data indicate that white bread protein is primarily deficient in the two essential amino acids, lysine and valine. When these are supplied in adequate amounts along with pure vitamin and mineral supplements, white bread without milk will support excellent growth.

In two instances we have attempted to increase the growth-promoting value of the supplemented white breads through the addition

of other pure amino acids. We first tried the addition of methionine, tryptophane, and leucine to a white bread supplemented with lysine, valine, riboflavin, salts, and A, D, E oil, but did not obtain better growth. We later added methionine, histidine, tryptophane, and isoleucine to a bread supplemented with 0.8% l(+)-lysine, 0.3% l(+)-valine, riboflavin, salts, and A, D, E oil. In this case, while we obtained better growth, the difference was not significant. The nutritive factors which restrict the growth of the animals fed these supplemented breads to less than the optimal rate, are not determined by our data.

White bread made without milk, supplemented with lysine and valine, requires additional riboflavin to support the maximum growth possible within the limitations of this diet. Other factors of the B complex appear to be present in sufficient concentration to permit excellent growth when the protein, salt, and riboflavin deficiencies have been corrected. However, the supply of these factors does not appear to be adequate for reproduction and lactation.

While our data do not indicate which minerals are deficient in white bread, the evidence is clear that certain constituents of the Osborne and Mendel salt mixture are needed to permit the full expression of the growth obtainable when the protein and vitamin limitations of white bread have been improved. Calcium is undoubtedly an important factor as shown by Henry's (1941) work and indicated by our data in Table II.

White bread of high nutritional value is produced when supplemented with natural food products, such as dry yeast or dry milk, which will contribute an adequate amount of lysine. One may also make use of low-fat soy flour to replace part of the white flour, since soy flour contains about 2.4% lysine.

Summary

The nutritive value of white bread was studied, using white rats which were fed a diet composed exclusively of bread supplemented with the fat-soluble vitamins A, D, and E. The results show that white bread made from relatively high patent flour and without milk is deficient in lysine, valine, riboflavin, and mineral salts.

For young growing rats a white bread made with 6% dry skim milk solids permitted good growth and apparent good health and is equal to whole wheat for promotion of growth.

The incorporation of 0.25% l(+)-lysine, 0.27% l(+)-valine, 2.0% Osborne and Mendel salt mixture plus supplementary vitamins A, D, and E, and 0.8 mg riboflavin per pound loaf produced a bread which,

when fed ad libitum, gave better growth than either a 6% milk bread, or a straight whole wheat bread.

Proteins supplying the amino acids, lysine and valine, are present in milk, yeast, and soybeans. These products, when added to bread in sufficient amounts, will correct the amino-acid deficiencies of white bread.

A white bread fortified with 0.25% lysine, 0.27% valine, 2.0% Osborne and Mendel salt mixture, vitamins A, D, and E, and 0.8 mg riboflavin per loaf is more than twice as efficient in producing weight gains in young white rats than either basic white bread or enriched white bread[6] and about one third more efficient than either whole wheat or 6% milk bread over an 8-week test period.

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[6] Enriched bread as used in this paper refers to enrichment levels specified prior to May 1942, and does not include milk.

A SURVEY OF THE VITAMIN AND MINERAL CONTENT OF BAKERS' CAKES AND PIES

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In a recent article by the Council on Foods and Nutrition of the American Medical Association (1942) attention was drawn to the progressively increasing consumption of sugar and of other relatively pure carbohydrates by the American public during recent years. Since this dietary tendency is regarded as a serious obstacle to improved national nutrition, the Council believes, "It would be in the interest of the public health for all practical means to be taken to limit consumption of sugar in any form in which it fails to be combined with significant proportions of other foods of high nutritive quality."

Some attempts have been made in this country to enrich sugar, especially with vitamin B_1, but no satisfactory solution to this problem has yet been reported. It seems therefore that the alternative of enriching foods like cakes, pies, and confections, in the manufacture of which a high proportion of sugar is employed, is a rational step in the same direction.

In this report are presented the results of a survey of the extent to which commercial cakes and pies furnish some of the more important nutritional elements. Suggestions are included as to which products might profitably be enriched, and the minimal standards for such enrichment.

Sampling

Representative samples of 50 different cakes and 50 different pies, baked in 53 bakeries were purchased on the retail market in New York, Chicago, Atlanta, Omaha, and Los Angeles. The samples were collected by agencies in no way connected with the baking industry. The purchases were made in middle- and low-income neighborhoods where it is claimed most cakes are bought.

The cakes were of four commercial types: (a) The foam type, such as sponge and angel food, in various shapes and sizes; (b) The light-batter type, including layer cakes of different sizes, with a variety of icings, and a number of cup cakes; (c) The heavy-batter type, such as pound cake, iced or un-iced; (d) Specialty goods containing fruits and/or nuts. The three main types of pies selected were apple, fruit pies other than apple, and a number of specialty items. The samples were standard 9-inch pies, each considered to consist of six

servings. The kinds of cakes and pies and their relative numbers reflect their popularity in the American market.

Analyses were conducted upon the aliquots of the ground, air-dried cakes and of the homogenized pies for vitamin A, thiamine, ascorbic acid, riboflavin, niacin, calcium, and iron content. Precautions were taken in preparing the composites to prevent contamination with minerals and loss of any nutrient. Analyses for all of the above factors were not conducted upon every sample. When a figure for a given nutrient is omitted from the tables it may be regarded as being present in this particular product [deductions from the tables compiled by Daniel and Munsell (1937) and Booher and associates (1942)] in quantities too low to justify the analysis.

Methods of Analysis

All the values are reported for the cakes and pies on the "as received" basis. Vitamin A was determined by the antimony trichloride procedure (Dann and Evelyn, 1938; Oser, Melnick, and Pader, 1943). Since the vitamin A in the test products was derived from butterfat and eggs, part of it was known to be present in the

TABLE I

VITAMIN AND MINERAL CONTENT OF BAKERS' CAKES

(Values expressed on the "as received" basis. Figures in parentheses indicate range)

Type of cake	Number samples tested	Values found, per 100 g				
		Thiamine	Riboflavin	Niacin	Calcium	Iron
		μg	*μg*	*mg*	*mg*	*mg*
Foam	7	31 (7–82)	150 (75–263)	0.54 (0.23–0.89)	69 (12–173)	2.0 (0.7–5.4)
Light batter	26	32 (5–65)	97 (35–171)	0.67 (0.34–1.12)	62 (35–117)	2.0 (0.7–5.1)
Heavy batter	10	40 (23–74)	92 (51–131)	0.72 (0.35–1.26)	48 (29–79)	1.5 (0.9–2.5)
Fruit or nut	7	60 (36–104)	96 (55–125)	0.66 (0.34–0.93)	51 (35–82)	2.3 (1.5–3.5)

form of the provitamin, carotene. Accordingly, separate tests for this factor were made by the colorimetric A.O.A.C. (1940) procedure. Thiamine was determined by the thiochrome procedure (Hennessy, 1941). Ascorbic acid was estimated photometrically (Bessey, 1938; Hochberg, Melnick, and Oser, 1943); only total ascorbic acid values (dehydro- plus reduced-ascorbic acid) are reported. Riboflavin was determined by the microbiological method (Snell and Strong, 1939;

Andrews, Boyd, and Terry, 1942), niacin colorimetrically (Melnick, 1942), calcium titrimetrically (A.O.A.C., 1940), and iron by the colorimetric procedure (A.O.A.C., 1940).

Results

The results of the present tests are summarized in Tables I and II.

Maximal, minimal, and average figures are presented for the different kinds of cakes and pies. The results of the analyses for

TABLE II

VITAMIN AND MINERAL CONTENT OF BAKERS' PIES

(Data refer only to standard 9-inch pies. Values expressed on the "as received" basis. Figures in parentheses indicate range)

Identity	Number samples tested	Average serving	Values found, per 100 g						
			Vitamin A	Thiamine	Ascorbic Acid	Riboflavin	Niacin	Calcium	Iron
		g	*USP units*	*μg*	*mg*	*μg*	*mg*	*mg*	*mg*
Apple	12	201 (183–227)	—	—	0.44 (0.0–1.1)	—	0.40 (0.21–0.55)	—	1.9 (1.2–3.0)
Pineapple	9	174 (148–210)	—	—	0.55 (0.0–1.0)	—	0.40 (0.24–0.55)	—	0.8 (0.7–0.9)
Cherry	8	181 (153–207)	—	—	0.43 (0.1–1.2)	—	0.46 (0.29–0.76)	—	0.8 (0.6–1.0)
Berry	8	174 (144–203)	—	—	0.37 (0.0–0.9)	—	0.43 (0.20–0.64)	—	1.0 (0.9–1.0)
Coconut custard	5	176 (151–214)	226 (171–257)	61 (58–66)	—	187 (109–218)	0.29 (0.27–0.30)	125 (120–130)	1.2 (0.9–1.4)
Lemon meringue	3	160 (134–187)	174 (92–256)	32 (30–34)	0.90 (0.6–1.2)	83 (78–88)	0.19 (0.18–0.20)	20	0.5
Mince	3	218 (186–251)	—	68 (65–70)	0.60	35 (26–44)	0.35 (0.29–0.40)	—	2.2
Pineapple cheese	1	185	376	93	0.80	220	0.36	50	0.7
Pumpkin	1	183	9830	—	0.30	—	0.50	90	1.0

different samples of the same type of product, even though purchased in the same city, were found to vary widely. Some samples which contained appreciable amounts of one nutrient were wanting in other factors.

Consideration of the data from the standpoint of average cake or pie should be undertaken with some reservation in view of the wide deviation of individual values within each series. However, in order to express a general opinion on the nutritional value of these products in the American dietary, it is permissible to base interpretations on average values. This decision is supported by the relatively large number of samples subjected to assay.

Interpretations and Recommendations

It is unreasonable to expect the values reported for the cakes and pies to compare favorably with figures in the literature for the protective foods such as meat and dairy products, fresh fruits, and vegetables, etc. Likewise one should not compare the results with values for other desserts commonly consumed, since many of these contribute very little in food value other than calories. Accordingly, the values obtained for the cakes and pies have been interpreted in absolute terms, dealing with the degree to which these foods furnish specific nutrients in proportion to calories. Justification for this approach may be found in the fact that it was one of the guiding principles used by the U. S. Food and Drug Administration in arriving at the standard levels for enrichment of flour and bread.

Under the present Federal Food, Drug, and Cosmetic Act (1941) the minimal daily requirements for the various vitamins and minerals for which assays were made in the present study, are:

Vitamin A	4000 USP units
Thiamine	1.0 mg
Ascorbic acid	30 "
Riboflavin	2.0 "
Niacin	10 "
Calcium	750 "
Iron	10 "

For the purpose of the present discussion it has been assumed that an individual consuming 2500 calories eats one serving of cake or pie during the day. It is considered desirable by some nutritionists that foods should furnish their specific nutrients at least in the same proportions to the daily requirements as they furnish calories. This criterion was used to show to what extent each kind of cake or pie conforms to such a theoretical ideal, *i.e.*, the relative "nutrient load" carried by the calories in a serving. These proportions hold regardless of the size of the serving since they are relative to calories. The results of this evaluation of cakes and pies are presented in Tables III and IV.

All the cakes furnish insufficient quantities of thiamine and niacin to the dietary. This is also true in the case of riboflavin and calcium; foam type cakes, however, carry their nutrient load of these factors to a significantly greater extent than the other three types of cake. All four classes of cake furnish a satisfactory amount of iron to the dietary. The data presented in Table III also suggest minimal standards for the enrichment of cake, if such a step is contemplated. The suggested minimal level involves enriching these foods so that they would carry their full nutrient loads. Under such circumstances

they would no longer be dependent upon other foods in the dietary to compensate for their deficiencies.

The nutritional evaluation of the pies is presented in Table IV.

The values for the most popular pies—apple, pineapple, cherry, and berry—are comparable but cannot be regarded as good; on the average only 16% of their nutrient load of ascorbic acid and 44% of that of niacin were furnished in one serving. However, these pies contribute satisfactory quantities of iron to the dietary.

Mince and lemon meringue are not significantly superior to the fruit pies from the nutritional point of view. They do, however,

TABLE III

NUTRITIONAL EVALUATION OF CAKES SOLD ON AMERICAN MARKET

Identity	Number samples tested	Nutritional values furnished by 100 g											
		Calories		Thiamine		Riboflavin		Niacin		Calcium		Iron	
		Total [1]	% of daily intake [2]	% M.D.R. [3]	% Nutr. load [4]	% M.D.R. [3]	% Nutr. load [4]	% M.D.R. [3]	% Nutr. load [4]	% M.D.R. [3]	% Nutr. load [4]	% M.D.R. [3]	% Nutr. load [4]
Foam	7	280	11	3	27	8	73	5	45	9	82	20	182
Light batter	26	360	14	3	21	5	36	7	50	8	57	20	143
Heavy batter	10	390	16	4	25	5	31	7	44	6	38	15	94
Fruit or nut	7	370	15	6	40	5	33	7	47	7	47	23	153

[1] The values in this column are based upon proximate analyses conducted on the individual samples. The data were furnished by Mr. R. B. Meckel, American Institute of Baking, Chicago, Ill.

[2] Total caloric intake is assumed to be 2500 calories per day.

[3] The percent of the minimal daily requirement of each nutrient contained in one serving of cake. The values in these columns are based upon the average figures in Table I and the values for the minimal daily requirement (Federal Food, Drug, and Cosmetic Act, 1941).

[4] The percent of the "nutrient load" (see text) of each nutrient, contained in one serving of cake.

furnish a larger number of essential food factors in quantities which have some nutritional significance.

Nutritionally speaking, pumpkin, coconut custard, and pineapple cheese pies must be regarded as the three outstanding pies. The former carries more than its nutrient load in the case of vitamin A,[1] calcium, and iron. Coconut custard pie furnishes satisfactory quantities of riboflavin, calcium, and iron, and significant quantities of vitamin A and thiamine. Pineapple cheese pie furnishes ample

[1] The vitamin A value of the pumpkin pie was found to be far in excess of that reported in the literature for the fresh whole vegetable. For this reason the carotene analysis was repeated, but this time the xanthophyll-free extract prepared according to the A.O.A.C. (1940) procedure was subjected to chromatographic adsorption on, and subsequent elution from, the magnesium oxide column according to the method of Fraps and Kemmerer (1941). No difference in the carotene value was obtained despite this improvement in the specificity of the assay procedure.

quantities of vitamin A, thiamine, riboflavin, and iron. Furthermore, these three types of pie furnish approximately 15 g of protein per serving, fully three times that yielded by the fruit pies. Since the proteins in the former pies are derived mainly from milk and eggs, foods characterized by the excellent biological value of their proteins, these pies must also be regarded as good sources of protein. Because of the factors described above these pies need not undergo enrichment.

TABLE IV

NUTRITIONAL EVALUATION OF PIES SOLD ON AMERICAN MARKET

Identity	Number samples tested	Nutritional values furnished by an average serving [1]															
		Calories		Vitamin A		Thiamine		Ascorbic acid		Riboflavin		Niacin		Calcium		Iron	
		Total [2]	% of daily intake [3]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]
Apple	12	540	22					3	14			8	36			38	181
Pineapple	9	360	14					3	21			7	50			14	100
Cherry	8	440	18					3	17			8	44			15	83
Berry	8	430	17					2	12			8	47			17	100
Coconut custard	5	380	15	10	67	11	73			15	100	5	33	29	193	21	140
Lemon meringue	3	350	14	7	50	5	36	5	36	7	50	3	21	4	29	8	57
Mince	3	660	26			15	58	4	15	4	15	8	31			48	185
Pineapple cheese	1	290	12	17	142	17	142	5	42	20	167	7	58	12	100	13	108
Pumpkin	1	400	16	450	2810			2	13			9	56	22	137	18	112

[1] The figures for the average serving of each kind of pie are given in Table II.
[2] The values in this column are based upon proximate analyses conducted upon representative pies.
[3] Total caloric intake is assumed to be 2500 calories per day.
[4] The percent of the minimal daily requirement of each nutrient contained in one serving of pie. The values in these columns are based upon the average figures in Table II and the values for the minimal daily requirement (Federal Food, Drug, and Cosmetic Act, 1941).
[5] The percent of the "nutrient load" (see text) of each nutrient, contained in one serving of pie.

In the case of cake there is justification for thiamine, riboflavin, niacin, and calcium enrichment. In connection with the last three nutrients, no technicological problem exists; riboflavin (Andrews, Boyd, and Terry, 1942) and niacin (Melnick, 1942) are stable during the baking process; calcium, of course, would be unaffected. Some preliminary experiments have been conducted to determine the possibility of enriching cake with thiamine. These tests were carried out on a commercial scale. The results are presented in Table V. The

levels of enrichment were purposely exaggerated in order to minimize possible lack of specificity and precision of the assay method as factors complicating interpretation of the results. The results indicate that cakes may be enriched as readily as bread, the thiamine loss being only slightly greater (Melnick and associates, 1941). This may be due to the fact that bread is more acid (average pH 5.8) than most cakes. It would seem from the tests recorded that the thiamine losses tend to be greater with increasing alkalinity of the batter.

In the case of fruit pies, the most logical and important factor which should be stabilized or restored is ascorbic acid. The low ascorbic

TABLE V

THIAMINE ENRICHMENT OF CAKE ON A COMMERCIAL SCALE
(Values expressed on the "as received" basis.)

Type of cake	pH of batter	Sample	Thiamine added [1]	Total thiamine found	Thiamine loss
			μg/100 g	μg/100 g	%
Foam	5.9	Basal	0	47	—
		Enriched	1040	1000	8
Foam	7.9	Basal	0	77	—
		Enriched	415	394	24
Light batter	7.3	Basal	0	63	—
		Enriched	454	376	31
Heavy batter	6.4	Basal	0	56	—
		Enriched	421	394	20
Heavy batter	7.1	Basal	0	61	—
		Enriched	380	356	22

[1] Synthetic thiamine was used as the means of enrichment.

acid values listed for these products are not surprising. Losses of as much as 90% of this vitamin have been reported in the baking of pie (Kohman, 1942). We have observed ascorbic acid to be more stable in small 4-inch pies which require a much shorter period of baking.

Summary

Fifty samples each of commercial cakes and pies were subjected to vitamin and mineral assays. In terms of calories furnished, "average" cake carries from one-fifth to four-fifths of its nutrient load of thiamine, riboflavin, niacin, and calcium. However, in the case of iron, ample proportions are supplied. Cake may be enriched as readily as bread. Except for iron, fruit pies, likewise, are dependent upon other items in the dietary to compensate for their nutritional deficiencies. The

custard and cheese pies furnish satisfactory quantities of vitamin A, thiamine, riboflavin, calcium, iron, and protein. Minimal standards for the enrichment of cake and pie are suggested.

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A COMPARISON OF BRABENDER EXPERIMENTAL AND COMMERCIAL MILL FLOURS WITH SPECIAL REFERENCE TO THEIR DIASTATIC ACTIVITY AND GASSING POWER VALUES

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The use of an experimental mill for the study of the properties of wheat flour presupposes a reasonable agreement between the experimental and corresponding commercial flour characteristics. Considering the importance of this point, remarkably little work has been published on the subject and what there is is frequently contradictory.

No great effort has ever been made to standardize the experimental milling test, though the necessity has been apparent for some time. Markley and Treloar (1937) in a study of the influence of 12 individual milling techniques on flour and loaf characteristics concluded that "there is need for further work upon the experimental milling technique in order to improve the forecasting of the ash, protein, and diastatic activities of commercially milled flours." The milling techniques used in that study were not described.

According to Ziegler (1940), the Milling and Baking Section of the Sixth International Technical and Chemical Congress of Agricultural Industries held in Budapest in July, 1939, passed a resolution that the standardization of the laboratory milling test should receive priority in discussions at the next congress, thus showing an appreciation of its importance.

The experimental mill systems vary considerably with respect to the number of break and reduction rolls and type of sifters used. Ziegler (1938) reviewed the more common systems which he gave as follows:

	Number of breaks	Reductions
Willard and Swanson (1911)	5	21
Geddes and West (1930)	5	8
Barbade (1934)	4	4
Brabender automatic mill (1935)	1	1
Kranz (1934, 1935)	3	3
Buhler automatic mill (1935)	3	3
Geddes and Aitken (1937) (Allis-Chalmers experimental mill)	5	8

Apart from the Brabender mill, there appears to be general agreement that the commercial-mill flours possess higher diastatic activity

than the experimental-mill flours and that a correlation exists between the two. In 1927 this point was emphasized by Blish and Sandstedt and later was discussed by Pascoe, Gortner, and Sherwood (1930) in their report of some extensive investigations on commercially milled vs. experimentally milled flours. The work of Markley and Bailey (1934), Swanson (1935), Leatherock, McGhee, and Giertz (1937), and Jones (1940) further supports the statement above.

In a survey of the literature, only one detailed reference (Shellenberger, 1938) to the gassing power and diastatic activity of experimental and commercial flours was found other than that by the author (Bottomley, 1938), though Cayzer and Jones (1938) compared the gassing power by means of the fermentograph of the experimental and commercial flours from two different types of wheat. Cayzer and Jones used an Allis-Chalmers type of experimental mill with four breaks and eight reductions. They gave no diastatic activity figures but showed that the commercially milled flours produced more gas on fermentation than did the corresponding experimentally milled flours. The difference was not, however, significant in their baking test, since 0.5% of malt flour was included in the baking formula. They suggested that the reason for the lower gassing power of the experimentally milled flour was that in the laboratory mill the roll pressure is not so great as in the commercial mill, and consequently one would expect a lower proportion of ruptured starch grains.

Shellenberger (1938) reported the results of a comparison of the diastatic activity determinations by the Blish-Sandstedt method and the gassing power by the Blish-Sandstedt manometric method of 104 commercially and 85 experimentally milled flours. The type of mill is not discussed but one assumes that it is of the Allis-Chalmers type since these are widely favored in America. He found that, on the average, the commercially milled flours had a greater hourly gassing rate than the experimentally milled flours and that the gassing power was sustained for a longer time. For both series of flours he found a significant relationship between diastatic activity and gassing power after all the initial sugars present in the flours had been fermented, that is, after three hours' fermentation under the conditions of his experiment.

The Brabender mill differs radically from the other types of experimental mill. In the first place it is a very short system mill, having only one break and one reduction. Further, these do not consist of pairs of corrugated and smooth rolls but of pairs of conical artificial stones operating in a horizontal plane. The wheat is fed to the upper pair of stones through a hopper and a variable gauge which controls the rate of feed. The partially ground stock passes to a sifter where

any bran and flour are directly removed to the bran and flour box and the middlings are passed on to the second pair of stones for reduction, after which the products are sieved on a second sifter. This separates the flour, pollard, and any further bran.

The sieves on the mill used in this investigation carry (1) a 20-wire and a 9xx silk and (2) a 7xxx and 7xx silk [1] for the break and reduction stock, respectively. The Brabender mill has been described in detail by Mueller (1934, 1935) and further reference to its design is not necessary here.

The comparative value of the Brabender automatic and Allis-Chalmers experimental mills for wheat investigations has been studied by Geddes and Aitken (1937). They found on making baking tests that a better differentiation between samples was obtained with the Allis-Chalmers flours and that these were closer in appearance to commercial-mill flours. It is pointed out, however, that the fundamental purpose of the Brabender mill is to supply in the shortest possible time a flour sample for farinograph testing (*i.e.*, for physical testing) and not primarily to yield flours comparable to those produced by commercial mills.

Geddes and Aitken did not present or discuss the diastatic activity or gassing power values of their samples. The flour extractions obtained by them on the Brabender mill I consider to be somewhat low, though they used sieves different from those described above. They obtained a mean flour yield of 47.3% and 67.9% for the Brabender and Allis-Chalmers milled samples, respectively. In the following study the flour yields for the Brabender-milled samples mainly varied from 58% to 62% and they are compared with corresponding samples milled on a commercial mill having an output of 5 tons of flour per hour, and a flour extraction of approximately 72%.

In addition to this series of comparisons, there is a second which shows the differences between Brabender-milled flours and flours from a Tattersall mill, which is one of the smallest commercial roller mills in use. It possesses two pairs of break and two of reduction rolls and two centrifugals, all of normal commercial dimensions, and is, therefore, a short-system mill. Under the conditions of this study it was set to give a flour extraction of 72% with an output of from 350 to 400 pounds of flour per hour.

The Tattersall mill is especially useful for studying the milling properties of different wheat varieties and for rapidly obtaining sufficient flour to enable commercial baking tests to be made, since it is only necessary to have from eight to ten bags of wheat. It is frequently impossible to obtain sufficient of a new wheat variety to

[1] A "20" wire has 20, a 9xx silk 97, a 7xxx silk 86, and a 7xx silk 82 meshes per lineal inch.

TABLE I

COMPARISON OF BRABENDER (B) AND CORRESPONDING COMMERCIAL (C) MILL FLOURS

(Extraction of commercial flours approximately 72%)

Sample	Mill	Extraction	Moisture	Protein (N × 5.7)	Absorption (farinograph)	Maltose	Ash	Gassing power (fermentograph)			
								Total CO_2 per		Maximum gas production, hour and amount	
								5 hours	7 hours		
		%	%	%	%	%	%	*ml*	*ml*	*hr*	*ml*
Commercial blend	1 B	59.8	14.8	6.95	54.1	2.01	—	2,245	—	4th	530
	1 C	—	14.0	6.90	56.7	1.70	—	2,010	—	4th	460
Commercial blend	2 B	61.0	13.5	7.87	56.3	2.60	—	2,440	3,230	3rd	550
	2 C	—	13.4	7.61	58.3	2.32	—	2,290	2,970	3rd	530
Commercial blend	3 B	59.0	14.1	8.12	56.5	2.01	0.524	2,035	2,385	4th	500
	3 C	—	13.4	7.81	59.1	1.83	0.396	1,915	2,405	3rd	450
Commercial blend	4 B	59.0	13.1	8.44	58.7	2.09	—	2,140	2,720	4th	530
	4 C	—	12.6	8.06	59.4	1.94	—	1,950	2,450	4th	450
Commercial blend	5 B	61.5	15.0	8.44	57.0	2.05	—	2,405	2,865	3rd	560
	5 C	—	14.4	8.44	60.3	1.83	—	2,160	2,620	3rd	510
Commercial blend	6 B	57.5	13.5	9.12	59.2	2.20	0.510	2,330	2,760	4th	570
	6 C	—	13.5	9.23	61.8	1.94	0.402	2,200	2,590	3rd	540
Commercial blend	7 B	58.8	14.1	9.40	59.7	2.18	0.504	2,390	2,860	4th	570
	7 C	—	13.0	9.46	62.3	2.01	0.402	2,260	2,670	3rd	530
Commercial blend	8 B	61.5	14.2	9.80	59.0	1.83	—	2,020	2,235	3rd	620
	8 C	—	13.5	9.75	60.0	1.49	—	1,710	—	3rd	550
Commercial blend	9 B	58.5	15.1	10.00	57.2	1.80	—	1,790	—	4th	540
	9 C	—	14.5	10.03	60.1	1.72	—	1,800	—	4th	530
Commercial blend	10 B	58.5	13.5	10.54	59.7	2.01	—	2,155	2,380	4th	590
	10 C	—	13.1	10.60	62.3	1.67	—	1,870	2,075	3rd	580
Commercial blend	11 B	58.5	15.5	11.06	60.4	2.95	—	2,350	2,800	3rd & 4th	540
	11 C	—	14.5	10.83	62.4	2.43	—	2,065	2,295	3rd	525
Commercial blend	12 B	62.0	13.7	11.23	62.1	2.28	—	2,060	2,280	3rd & 4th	570
	12 C	—	13.3	11.34	64.2	1.83	—	1,790	1,960	3rd	550
Commercial blend	13 B	60.5	15.1	12.08	63.6	3.03	0.668	2,360	2,670	4th	610
	13 C	—	13.1	11.91	67.5	2.43	0.458	1,910	2,150	3rd	490
Commercial blend	14 B	60.0	15.1	12.77	64.5	3.20	0.656	2,360	2,850	4th	580
	14 C	—	14.1	12.43	65.2	2.48	0.450	2,050	2,270	3rd	530

Commercial blend	^{14}C	—	14.1	12.43	65.2	2.48	0.456	2,650	2,975		

TABLE II

COMPARISON OF BRABENDER (B) AND CORRESPONDING TATTERSALL (T) MILL FLOURS

(Extraction of Tattersall mill flours approximately 72%)

Sample	Mill	Extraction	Moisture	Protein (N × 5.7)	Absorption (farinograph)	Maltose	Ash	Gassing power (fermentograph)			
								Total CO_2 per		Maximum gas production, hour and amount	
								5 hours	7 hours		
		%	%	%	%	%	%	*ml*	*ml*	*hr*	*ml*
Blend 10/38	B	60.0	13.7	9.63	59.2	2.43	0.542	2,280	2,600	4th	560
	T	—	13.5	9.75	60.9	1.80	0.492	2,090	2,385	3rd	540
Blend 12/38	B	61.5	13.8	9.58	56.8	1.62	—	1,905	2,090	3rd	560
	T	—	13.3	9.46	58.5	1.34	—	1,780	1,960	3rd	555
Dundee 9/38	B	55.0	14.6	10.03	62.7	4.26	0.714	2,140	2,920	4th	500
	T	—	13.6	9.58	65.6	3.73	0.550	1,980	2,675	3rd & 4th	460
Dundee 3/39	B	61.8	14.3	15.25	64.4	2.98	0.768	2,190	2,370	3rd	590
	T	—	13.5	15.22	67.8	2.66	0.598	1,820	1,960	3rd	610
Ford 9/38	B	62.5	14.5	10.37	52.4	1.33	—	1,440	1,560	3rd	450
	T	—	14.1	10.32	54.5	1.07	—	1,220	1,310	3rd	410
Ford 3/39	B	64.5	13.5	12.14	56.6	1.47	—	1,380	1,480	3rd	390
	T	—	13.1	11.96	56.6	1.14	0.446	1,050	1,160	3rd	320
Pusa 8/39	B	57.0	16.1	14.59	60.5	2.53	—	2,510	2,870	4th	710
	T	—	13.8	14.65	66.3	2.37	—	2,240	2,490	4th	620
Blend 7/41	B	60.0	14.2	10.72	59.7	1.87	—	2,215	2,505	4th	625
	T	—	13.0	10.83	65.0	1.80	—	2,210	2,530	3rd & 4th	610
Ranee 3/41 (Longerenong)	B	60.0	13.7	10.03	60.8	2.13	—	2,720	3,180	3rd & 4th	640
	T	—	13.5	10.09	62.3	1.70	0.396	2,315	2,785	3rd	555
Baldmin 6/41 (Longerenong)	B	67.5	14.9	10.49	60.1	3.39	0.574	2,380	3,010	4th	620
	T	—	13.6	10.43	51.2	1.97	0.500	1,995	2,225	4th	560
Ghurka 3/41 (Longerenong)	B	60.5	14.1	11.00	59.7	1.45	—	1,415	—	3rd	475
	T	—	13.7	11.17	62.6	1.45	—	1,445	—	3rd	500
Magnet 3/41 (Longerenong)	B	61.5	13.9	11.86	59.1	1.65	—	2,165	2,240	3rd	700
	T	—	13.6	11.91	61.6	1.53	0.432	1,585	1,655	3rd	590
Dundee 6/41 (Longerenong)	B	65.0	14.3	13.57	64.4	3.29	0.616	2,750	3,045	4th	820
	T	—	13.6	13.05	65.3	2.22	0.540	2,110	2,280	3rd	660

enable a full-scale commercial milling to be made; furthermore such tests interfere with normal production.

Though we were chiefly concerned with diastatic activity and gassing power values, it was thought that it would be advantageous to other workers who are unable to make direct comparisons between their experimental equipment and commercial mills to present as full a comparison of the various flours as was possible. Consequently, all available analytical data are listed in the tables given below.

Methods

Diastatic activity was determined by the Kent-Jones (1939) method and the gassing power by the fermentograph (Brabender, 1935). In the final columns of Tables I and II are given the maximum values of the hourly gas production and the times at which they occurred. Moisture, protein, and ash were determined as follows:

Moisture, by drying overnight 5-g samples in an oven at 102°–103°C
Protein (N × 5.7), by the Kjeldahl method using 7 g potassium sulfate and a trace of copper sulfate to aid the sulfuric acid digestion
Ash, by igniting 5-g samples for 2 hours at 600°C in a muffle furnace.

The water absorptions of the flours as found by means of the farinograph are also listed.

The results have purposely not been calculated to a uniform moisture content basis, because the values obtained are informative. They show how much more moisture has been lost in the commercial, as compared with the experimental milling for the various types of wheat.

The wheats ground on the Brabender mill were running samples from the feeds to either the commercial or the Tattersall mill and, therefore, had been subjected to the commercial cleaning and tempering (damping) treatments. The commercial mill samples were also heat-conditioned but this was not possible for the Tattersall-mill samples. The flours from the commercial and Tattersall mills were running samples corresponding to the wheat samples. All flours were kept in air-tight tins during the course of the investigation.

Results

A comparison has been made of Brabender (B) and corresponding commercial (C) mill flours from 14 wheat blends which varied in protein content from 7% to 12½%. These samples were representative of blends varying from soft wheats suitable for the manufacture of biscuit flour to wheats appreciably harder and more proteinous than average Victorian wheats. The results are shown in Table I.

The comparison between the Brabender (B) and corresponding Tattersall (T) mill flours has been made for 13 samples covering 7 different wheat varieties and 3 commercial wheat blends. The protein content of these flours varied from 9½% to 15¼%. The results are listed in Table II. In order to illustrate in more detail the differences in gassing power between corresponding samples from different mills, the hourly production of carbon dioxide, as determined by the fermentograph, has been given in Table III for a number of samples taken from Tables I and II.

TABLE III

DIASTATIC ACTIVITY AND GASSING POWER OF CORRESPONDING EXPERIMENTALLY AND COMMERCIALLY MILLED FLOURS

(B = Brabender; C = commercial; T = Tattersall mill flour)

Sample flour	Fermentograph gassing power—ml CO_2 produced per hour											
	2		6		10		13		Blend 12/38		Ranee 3/41	
	B	C	B	C	B	C	B	C	B	T	B	T
1st hour	400	420	360	340	320	320	320	250	360	360	380	340
2nd hour	470	460	420	410	490	430	410	370	420	405	500	480
3rd hour	550	530	550	540	575	580	560	490	560	555	640	555
4th hour	540	480	570	510	590	400	610	460	410	320	640	520
5th hour	480	420	430	400	180	140	460	340	145	140	560	420
6th hour	420	350	270	250	125	110	180	140	105	100	350	280
7th hour	370	230	160	140	100	95	130	100	80	80	210	195
5-hour total	2440	2290	2330	2200	2155	1870	2360	1910	1905	1780	2720	2315
7-hour total	3230	2970	2760	2590	2380	2075	2670	2150	2090	1960	3280	2785
Diastatic activity, maltose % (Kent-Jones method)	2.60	2.32	2.20	1.94	2.01	1.67	3.03	2.43	1.62	1.34	2.13	1.70
Protein % (N × 5.7)	7.87	7.61	9.12	9.23	10.54	10.60	12.08	11.91	9.58	9.46	10.03	10.09

Discussion of Results

It will be seen from Tables I and II that the experimentally milled flours gave higher diastatic-activity values and, with one exception, higher gas-production values than did corresponding commercially milled samples. These differences tended to increase with increase in protein content of the samples as is shown in Table IV.

An important point in this connection is that although the gas production during fermentation is greater in quantity and is sustained for a longer time in the experimentally milled flours, the hourly rates of gas production are approximately paralleled by the commercially milled flours. Thus corresponding flours have similar gas production characteristics.

The differences in gas production values have been calculated from

the total gas produced (fermentograph method) in 5 hours, since this is more significant than the 7-hour total under the conditions of this experiment.

For all practical purposes the protein content of the experimentally milled flours is not significantly different from that of corresponding commercial flours. Without correcting the analytical data for differences in moisture content the maximum difference was 0.38%, the Brabender flour being the greater. The tendency for the experimental flours to have a slightly greater protein content is probably due to the unavoidable presence of small branny particles, which also accounts for the much higher ash figures. The latter increase markedly with increase in protein content and give little indication of the probable ash content of the commercial flour.

As is to be expected from the use of short-system milling, less moisture loss occurs in the experimental mill than in the longer commercial

TABLE IV

DIASTATIC ACTIVITY AND GASSING POWER IN RELATION TO PROTEIN CONTENT

Samples		Diastatic activity			Gassing power, CO_2 5-hour total		
No.	Protein range	Av	Min	Max	Av	Min	Max
	%	%	%	%	*ml*	*ml*	*ml*
14	7 to 12½	0.33	0.08	0.72	220	−10	450
9	7 to 10	0.22	0.08	0.34	164	−10	310
5	10½ to 12½	0.66	0.34	0.72	320	270	450

process. The greatest difference between corresponding samples is noted in the harder, higher-protein wheats, where it reached 2%.

The water absorption of the flours as determined by the farinograph was lower in the experimental than in the commercial flours by from 0.7% to 3.9%. While this difference is partially due to differences in moisture content, it is thought to be due mainly to differences in granulation between the flours.

The foregoing remarks apply very closely to comparisons between the experimental and corresponding Tattersall mill samples. In this instance the diastatic-activity values of the experimental flours were greater than those of the Tattersall-mill flours by an average of 0.36% (minimum 0, maximum 1.07%) and the gassing power values greater by an average of 292 ml CO_2 (minimum −30, maximum 640) per 5 hours.

It is not feasible to give similar figures for different protein levels, because the wheats ground differed so widely in type and differences

between varieties would be greater than differences between protein contents (*cf* Ford and Baldmin varieties).

Conclusions

It is not to be expected that an exact duplication of the performance of a commercial mill can be obtained by a small experimental mill. Consequently there must be always an interpretation of the experimental results by the operator. From the data presented it can be concluded that such an interpretation with regard to diastatic activity and protein content is possible by the use of the Brabender automatic laboratory mill.

The diastatic activity of flours from this mill is higher than that of corresponding commercial mill flours, which is the reverse of the results found by the use of other experimental mills. This difference increases markedly when the protein content of the samples is higher than 10%. There is, however, a definite similarity in the gas-production characteristics during fermentation, so that a substantially accurate forecast can be made of the gassing power of a commercial flour after consideration of the experimental results.

The differences in diastatic-activity and gassing-power values between the two sets of flours is less in the series of samples of wheat blends than in the series covering different varieties. This indicates that it would be more difficult for the wheat breeder to interpret results than for the commercial cereal chemist who is more concerned with blends than individual wheat varieties.

There is only a very general relationship between diastatic activity (maltose figure) and gassing power (fermentograph) as was also found in an earlier study (Bottomley, 1938).

Although the protein content of corresponding experimental and commercial flours is approximately the same, the ash content varies widely. It is to the contamination of the experimental flours by bran and germ particles, as evidenced by the high ash figures, that the poor baking qualities of the Brabender-mill flours are ascribed. It is also probable that this contamination accounts to some extent for the poorer farinograph curves given by the experimental flours. Though not discussed earlier, it can be mentioned here that the farinograph curves of corresponding flours are similar in type but that the experimental flours always give a curve of less strength and stability than that given by the commercial flour.

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A FURTHER COMPARISON OF FLOURS OBTAINED WITH THE MICRO AND ALLIS-CHALMERS MILLS [1]

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Micro milling and baking methods have received attention from cereal researchers from time to time because of the relatively small quantity of wheat required for a test, as compared with the Allis-Chalmers and Buhler mills. Geddes and Frisell (1935) described a micro experimental flour mill, and comparisons of the chemical and baking properties of flours produced on the micro and Allis-Chalmers mills were made by Geddes and Aitken (1935) and Harris and Sanderson (1939). High positive correlations were obtained by both laboratories for flour protein and diastatic activity between the two sets of flours, but rather low correlations for flour yield and loaf volume were found by Harris and Sanderson in contrast to the highly significant relationships obtained by Geddes and Aitken. No correlation between flour ash on the two mills was discovered in the second study.

McCluggage (1943) reported the results of a study conducted with flours comparatively milled from hard red winter wheats by the Buhler and a Hobart laboratory grinder in which the latter was substituted for the usual experimental rolls. A flow similar to that commonly used in the Allis mill was employed, and the sifting was done on a Roto-matic sifter. Very high relationships were found between the loaf volumes of the flours milled by the two methods and likewise between wheat and Hobart flour protein and protein content and loaf volume. Approximately 300 g of wheat were required for each milling by the proposed micro method.

It was felt by the authors that the previous work of Harris and Sanderson (1939) should be repeated with special attention to milling yields on the two mills, and ash contents and relative baking quality of the two sets of flours. A different micro flow sheet from that employed by Harris and Sanderson is now in use in this laboratory and dissimilar results would naturally be expected. The micro technique is of marked importance at this station because of the substantial number of samples from the wheat nursery which it is necessary to handle each year in order that the breeding and propagation of new and desirable varieties will not be delayed. It has served to indicate baking quality from 2 to 3 years before determinations with the Allis

[1] Published with the approval of the Director of the Experiment Station.

procedure could be made The micro mill used is modeled after the one described by Geddes and Frisell (1935)

Material and Methods

In an initial experiment 25 replicate millings from a sample of high-grade hard red spring wheat were made on the Allis and micro mills. In a second study, 30 samples of different varieties of hard red spring wheat varying in test weight from 57.6 to 64.0 pounds per bushel were milled. The samples were prepared for milling by the usual procedure. The same operator did all millings on both mills, using 2000 g for the Allis and 175 g for the micro.

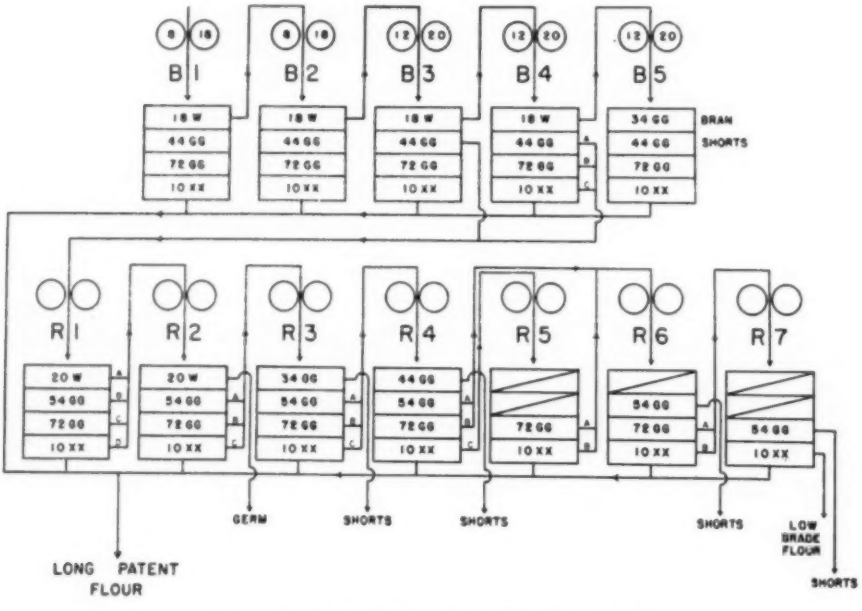

Fig. 1. Flow sheet used with the Allis-Chalmers mill.

Figure 1 shows the flow sheet employed for the Allis mill. Two stands of break rolls and one of reduction rolls were used. In break 4 the stock on 18 W went to break 5, while the material off the remaining sieves went to the first reduction, each classification being reduced separately, using an entirely different roll setting for the stock from each sieve. The letters A, B, etc., are used to designate the different reduction roll settings. The settings were not rigidly fixed to correspond to any particular letter but were adjusted according to the "feel" and appearance of the ground stock. After sifting break 5 stock the material on 34 GG and 44 GG was discarded as bran and

shorts, respectively; the two remaining sieves were left in the bolter, and two additional sieves, 20 W and 54 GG, were added. The stock from the several settings of reduction 1 were bolted as shown in the flow sheet.

The same procedure was followed in reducing the middlings from the various sieves after reduction 1, each separation being ground at a different roll setting. This procedure corresponds to commercial practice, where an effort is made to grind middlings of similar size in each reduction. When the end of the system was approached at reduction 7 the material on 54 GG was designated as "fine shorts"

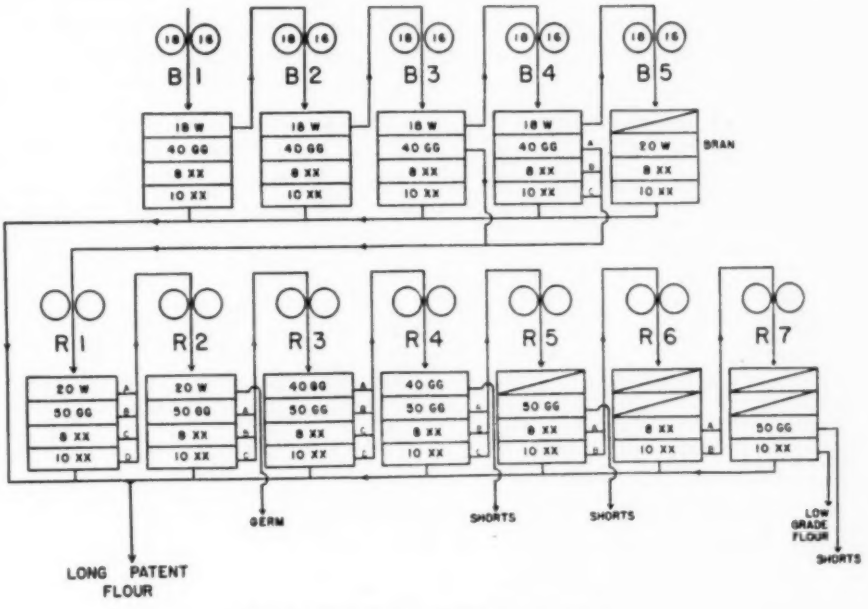

Fig. 2. Flow sheet used with the micro mill.

and discarded. Milling was continued until the material on 10XX matched in color and speckiness a standard sample of low-grade flour. In the case of some varieties an additional reduction was found necessary to obtain satisfactory results.

The flow sheet used with the micro mill method is very similar, as shown in Figure 2. Minor changes were made in some of the scalping sieves and an 8 XX sieve was used throughout in place of 72 GG. The technique employed, however, was identical with that used with the Allis-Chalmers mill with the exception that in the latter reductions a definite uniform roll setting was required owing to the limited amount of stock. The long-patent flour produced on the two mills represented

approximately 95% extraction of the total flour and was used for chemical and baking studies. In the second series of millings less pressure was used on the micro rolls with the aim of reducing the yield and producing a lower ash flour that would compare more favorably with that obtained on the Allis mill. The results were rather encouraging, and a further study of the comparative performance of the two mills was undertaken, employing different wheats in lieu of replicates of the same wheat.

The two sets of long-patent flours were baked by a 25 g micro baking method to enable a comparison to be made regarding their loaf volume and crumb color score. The Allis flours were also baked by a 100 g method to have a comparison between Allis flours baked in the usual manner and micro-milled and micro-baked flours from the same wheats. The malt-phosphate-bromate formula was employed with 5% sucrose (Harris 1939). A 3-hour fermentation period was used. The 25 g flour doughs were mixed in a Hobart mixer, using special dough hooks, while the 100 g flour doughs were mixed in the Hobart-Swanson mixer.

Results and Discussion

The data secured from the initial experiment will not be presented. It was found that the micro mill gave significantly higher yields of long-patent and total flour, but a lower yield of low-grade. The ash content of the micro flours was significantly higher, and the moisture content lower. These results agree, save for flour yield, with the work of Harris and Sanderson (1939).

The data for the second series of millings are summarized by the statistical constants shown in Table I. The flour yields for the two mills compared very favorably; the means and standard deviations are not statistically different. Flour ash was still significantly higher for the micro mill, though there was less difference between the means than in the initial series. The micro mill flour ash was somewhat more variable than the Allis, as might be expected.

No significant differences in mean loaf volume and variance were found. Crumb color scores were also included in the statistical treatment, although color measurement is a subjective determination. There was less agreement between milling methods in regard to color than in loaf volume, the micro flour showing greater color score variability.

Correlation coefficients showing the relationships between the two sets of millings in terms of the values studied are given in Table II. Flour yields are highly correlated. Allis flour yield can be predicted

TABLE I

STATISTICAL CONSTANTS CALCULATED FROM SERIES 2 MILLINGS

	Maximum		Minimum		Mean			Standard error		Standard deviation		Coefficient of variation	
	Allis	Micro	Allis	Micro	Allis	Micro	Difference [1]	Allis	Micro	Allis	Micro	Allis	Micro
Flour yield, long patent %	73.1	72.5	63.2	62.4	69.1	68.7	0.4	0.460	0.481	2.52	2.63	3.64	3.83
Flour yield, low grade %	5.2	5.0	2.6	2.6	3.4	3.4	0.0	0.113	0.108	0.62	0.59	18.10	17.29
Flour yield, total %	76.0	75.8	67.4	66.4	72.5	72.2	0.3	0.450	0.459	2.46	2.52	3.40	3.48
Flour ash %	0.49	0.59	0.36	0.39	0.409	0.476	**0.067**	0.006	0.008	0.033	0.045	0.80	0.94
Loaf volume cc	230	205	135	125	172.0	172.7	0.7	3.92	3.38	21.47	18.52	12.48	10.72
Loaf crumb color	8.5	8.5	5.5	5.0	7.60	7.48	0.12	0.146	0.140	0.80	0.77	10.53	10.28
Loaf volume, Allis 100 g, micro 25 g, cc	790	205	545	125	654.8	172.7	**482.1**	12.695	3.38	69.53	18.52	10.62	10.72
Crumb color, Allis 100 g, micro 25 g	8.5	8.5	6.0	5.0	7.27	7.48	0.21	0.096	0.140	0.53	0.77	7.27	10.28

[1] Significant differences are shown in heavier type.

from micro flour yield by use of the regression formula; Allis flour yield = 7.44 + 0.90 x micro flour yield. The correlation between the two series of ash contents is high, and wheat that yields a distinctly high ash flour with the micro mill would tend to give high values by the Allis method. Coefficients between test weight per bushel and flour yield were very significant, and not significantly different in magnitude between the mills. Baking results from the two sets of flours were significantly correlated, and showed that flours tend to give much the same baking results regardless of whether they are milled on the Allis

TABLE II

CORRELATION COEFFICIENTS COMPUTED FROM THE DATA OF SERIES 2 MILLINGS
(Value of r_{xy} at 5% point = 0.371)

Variables correlated		Correlation coefficient
X	*Y*	
Allis long-patent flour %	Micro long-patent flour %	+0.938
Allis low-grade flour %	Micro low-grade flour %	+0.793
Allis total flour %	Micro total flour %	+0.852
Allis long-patent flour ash %	Micro long-patent flour ash %	+0.756
Test weight lb/bu	Allis total flour %	+0.750
Test weight lb/bu	Micro total flour %	+0.800
Allis long-patent flour loaf volume cc	Micro long-patent flour loaf volume cc	+0.887
Allis long-patent flour crumb color	Micro long-patent flour crumb color	+0.833
Allis long-patent flour 100 g loaf volume cc	Micro long-patent flour 25 g loaf volume cc	+0.835
Allis long-patent flour 100 g crumb color	Micro long-patent flour 25 g crumb color	+0.688
Wheat protein %	Allis long-patent flour loaf volume (100 g) cc	+0.689
Wheat protein %	Allis long-patent flour loaf volume (25 g) cc	+0.588
Wheat protein %	Micro long-patent flour loaf volume (25 g) cc	+0.627

or micro mills. Loaf volume also tended to increase with wheat-protein content.

A higher relationship in baking strength between Allis- and micro-milled flour was found than in the work reported by Harris and Sanderson (1939), and in the present investigation a significant correlation for flour ash was shown, as contrasted with the previous findings. These results are in essential agreement with the conclusions of Geddes and Aitken (1935), and confirm the confidence placed in micro milling and baking methods.

Summary and Conclusions

A comparative study was made of milling yields, flour ash, loaf volume, and crumb color obtained from two series of hard red spring

wheat millings on the Allis-Chalmers and micro experimental mills. The millings were conducted in a laboratory with temperature and humidity control.

The first series comprised 25 replicate millings of one wheat on each mill. The micro mill gave higher yields of long-patent and total flour than the Allis mill. Flour ash was higher in the micro-milled flours, and the moisture content lower.

In the second series, 30 samples of different hard red spring wheat varieties were milled on the two mills. Less pressure was employed on the micro reduction rolls than in the first series with the result that flour yields and flour ash contents were in better agreement. No significant differences in flour yield were found between milling methods but micro flour ash, though reduced, was still higher than the Allis values.

High positive correlations were found for flour yields, flour ash, and loaf volumes between the two mills. As pointed out by other workers, a fairly reliable knowledge of milling and baking performance may be secured by micro milling and baking tests.

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FACTORS INFLUENCING THE PEARLING TEST FOR KERNEL HARDNESS IN WHEAT [1]

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Testing wheat for kernel hardness dates back almost to the beginning of milling. In early milling literature, the primitive test of chewing the wheat was described as a method of determining kernel hardness. One of the leaders in the movement to improvise more satisfactory tests for this characteristic was the Kansas Agricultural Experiment Station. Roberts (1910) developed a testing machine in which the wheat kernel was crushed by weighting an arm with an increasing load. The weight required to crush the wheat kernel was taken as an index of the kernel hardness. Unfortunately, this test was tedious, as a large number of kernels of each sample of wheat had to be tested in order to obtain reliable results, and because of the time required it was never used for large-scale testing.

The test developed at the Kansas Station was modified in Russia and other European countries by redesigning the equipment, according to Jelinek (1927), so that several kernels could be tested at once. Jelinek also reported results obtained by determining the cutting resistance of the kernels. He found a good relationship between the kernel hardness and the yield of total flour.

The pearling test for the determination of kernel hardness of wheat was developed by Taylor, Bayles, and Fifield (1939). In their work they adopted a procedure for making the test and proceeded to evaluate wheat varieties grown under different environments.

The pearling test is an adaptation of a test used by federal grain supervisors in grading barley for malting purposes. Essentially it consists of placing a weighed amount of wheat in a barley pearler, which is merely a carborundum wheel running in a closed case. After the machine has been run for a definite length of time, the pearled wheat is removed from the machine and weighed. It has been found that the harder the wheat kernel the less the amount of material removed in pearling. This test is rapid and accurate and requires little material.

The work reported in this paper deals with two phases of the pearling problem: The first part is a study of the pearling test as made under

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the agricultural experiment stations of the Great Plains region. Department of Milling Industry, Kansas Agricultural Experiment Station, contribution No. 81.

[2] Formerly agent, Division of Cereal Crops and Diseases. The writer acknowledges the assistance of Willard H. Meinecke, who performed the details of the experimental work described in this paper and assisted in designing many of the experiments reported.

various conditions; the second part is a presentation of an accumulation of data on kernel hardness for varieties of hard red winter wheat grown in the southern Great Plains in 1938, 1939, and 1940 and tested by the pearling technique developed as the result of the experiments described in the first part.

Material and Methods

The following equipment was used in the work reported herein: Strong-Scott barley pearler, model 38 without timer, equipped with a No. 30 grit stone; Cenco triple-beam balance sensitive to 1 cg; stop watch; one sieve covered with No. 20 wire. To provide the various speeds required in a part of the study, the pearler was driven by a belt and a variable-pitch pulley. Wherever the standard speed (1,725 rpm) was used the motor was connected directly to the drive shaft of the pearler. For most of the work the pearler was equipped with a 10-mesh wire screen made of wire 0.041 inch in diameter, Tyler code "Fijor." In one experiment this screen was replaced with a metal blank with two rows of holes punched along the edges.

The basic technique used in these studies was as follows: (1) Each charge (20 g) of the cleaned, unsized wheat was mixed thoroughly. (2) The charge was placed in the machine with the stone running at full speed; 60 seconds later the slide outlet was opened; and 10 seconds later the motor was stopped. (3) The pearled wheat was sifted over the 20-wire screen to remove dust and powdered material. The weight of the material remaining on the screen was recorded as the weight of pearled wheat.

In several experiments some of these details were varied because of the factor being studied. These variations will be indicated in the discussion of the results.

The effects of the following factors on pearling were studied: (1) temperature of the wheat and the pearler, (2) sifting of the pearled wheat, (3) speed of the stone and the length of the pearling time, (4) size of the charge, (5) the screen used in the pearler; and (6) moisture content of the wheat. Finally, the reliability of the pearling test was shown by comparing the results obtained in different laboratories on check samples.

Effect of Temperature of Wheat and Pearler

In this experiment the basic technique was used. Two samples of wheat were pearled under three sets of conditions: (1) wheat and pearler at room temperature, (2) pearler at room temperature and the wheat at low temperature (approximately 50° F), and (3) the wheat and the pearler at low temperature. Ten replicates of each of the samples

were pearled under each of the conditions mentioned. The data are summarized in Table I.

The implication of the data in Table I is that the pearling test is not sensitive to wide ranges in temperature. This means that no elaborate system of temperature control is required to reproduce the same pearling results from day to day and that the ordinary variations of room temperature should have little effect upon the results obtained.

TABLE I

EFFECT OF TEMPERATURE OF WHEAT AND MACHINE UPON THE AMOUNT OF WHEAT PEARLED OFF

Item	Wheat pearled off with—		
	Wheat and machine at room temperature	Wheat cold[1] and machine at room temperature	Wheat and machine cold[1]
	%	%	%
Sample No. 39920:			
Lowest value	36.2	35.6	34.9
Highest value	35.1	34.9	33.4
Range	1.1	.7	1.5
Mean	35.7 ± .1	35.3 ± .1	34.2 ± .2
Sample No. 39910:			
Lowest value	43.0	42.4	40.1
Highest value	41.3	41.4	39.6
Range	1.7	1.0	.5
Mean	42.2 ± .2	41.9 ± .1	39.9 ± .05
Mean difference between samples	6.5	6.6	5.7

[1] Approximately 50°F.

Effect of Sifting the Pearled Wheat

It was suggested that time and effort would be saved if the sifting of the pearled wheat over the 20-wire screen were omitted from the technique. In an experiment 21 subsamples of each of two varieties of wheat were pearled by the regular procedure, except that the pearled wheat was weighed before and after sifting on the 20-wire screen. The pertinent data obtained are presented in Table II.

Sifting the pearled wheat slightly increased the standard deviation for one variety and reduced it for the other. The difference between varieties was substantially the same in both cases. General experience with the pearling test indicates that there is less difficulty in obtaining weights that check from the various replications when the pearled wheat is sifted. This is an indication that sifting increases the accuracy of the pearling test.

TABLE II

EFFECT OF SIFTING PEARLED WHEAT UPON ACCURACY OF PEARLING TEST

Sample	Wheat pearled off	Standard deviation	Coefficient of variation	t
	%	%		
Unsifted Chiefkan	36.8	±.46	1.25	—
Unsifted Denton	61.2	±.71	1.16	—
Difference	24.4	±.60	—	40.67
Sifted Chiefkan	37.8	±.48	1.27	—
Sifted Denton	62.3	±.62	.99	—
Difference	24.5	±.55	—	44.54

Effect of Speed of Stone and Length of Pearling

The material used for this study consisted of five varieties of wheat; three of these covered the range of hardness ordinarily found in hard winter wheats, and the other two covered the range of hardness common with soft winter wheats.

The stone of the pearler was driven by a belt and an adjustable-

TABLE III

PERCENTAGE OF WHEAT PEARLED OFF AFTER 1, 2, AND 3 MINUTES WITH THE PEARLER RUNNING AT 1,725, 1,500, AND 1,300 RPM

Rate of speed and variety	Wheat pearled off in indicated time (minutes)		
	1	2	3
	%	%	%
1,725 rpm:			
Chiefkan	35.1	61.3	79.2
Kharkof	36.5	64.0	83.0
Blackhull	46.2	77.0	92.0
Trumbull	56.6	87.5	(1)
Dawson	61.5	90.0	(1)
Mean	47.2 ± .01	75.6 ± .015	—
Range	26.4	28.7	—
1,500 rpm:			
Chiefkan	21.5	39.2	51.5
Kharkof	21.3	39.6	55.4
Blackhull	26.0	48.7	65.7
Trumbull	32.2	59.8	78.0
Dawson	33.8	63.5	80.5
Mean	27.0 ± .005	50.2 ± .01	66.2 ± .01
Range	12.3	24.3	29.0
1,300 rpm:			
Chiefkan	13.1	24.7	35.8
Kharkof	13.4	26.3	37.1
Blackhull	16.0	31.6	46.5
Trumbull	19.3	39.8	56.1
Dawson	21.0	42.6	59.3
Mean	16.7 ± .005	33.0 ± .015	47.0 ± .01
Range	7.9	17.9	23.5

[1] The amount pearled off was so great that no accurate determination could be made.

pitch pulley. In this way any speed could be obtained by merely changing the pitch of the pulley. Preliminary experimentation revealed that approximately the same results were obtained at the three

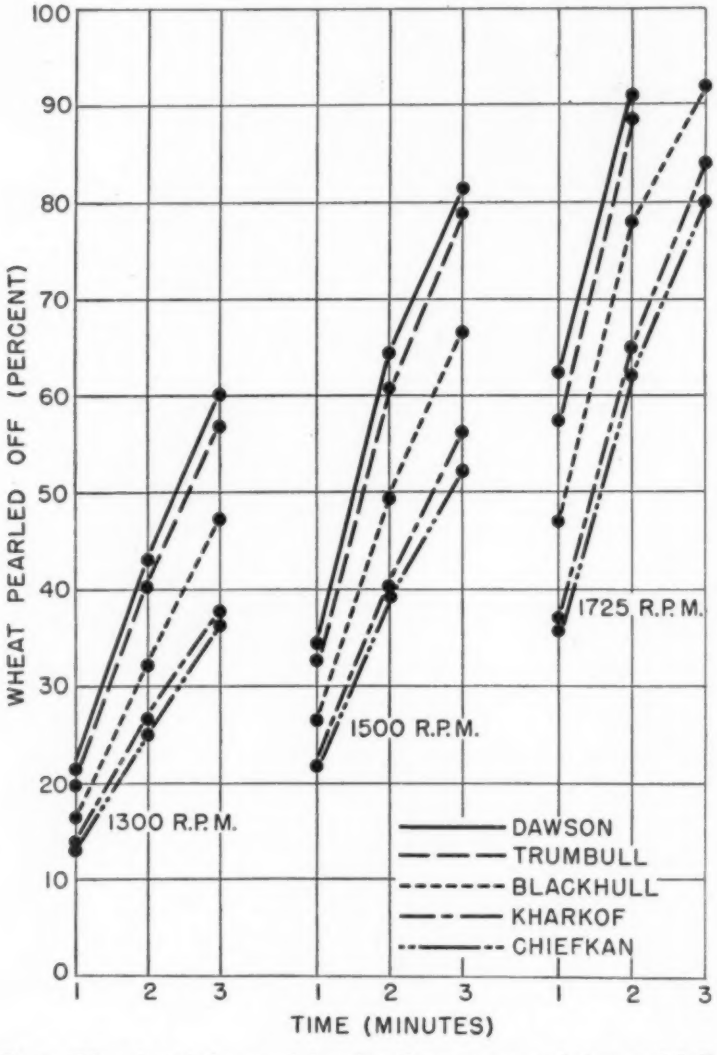

Fig. 1. Effect of speed of stone and time of pearling upon amount of wheat pearled off.

different speeds (1,725, 1,500, and 1,300 rpm) by pearling for 1, 2, and 3 minutes, respectively. Therefore, each of the five varieties was pearled under each of the nine possible combinations of speed and time. Fifteen replicate determinations were made for each variety under each set of conditions.

It is evident from Table III and from Figure 1, in which the data are plotted, that each combination of conditions (speed and time) gave a different level of results. The errors for the different means were approximately the same, however, indicating that the accuracy of the results was nearly the same whichever set of conditions was used.

The curves depart slightly from linearity, especially those for the higher speeds, owing, no doubt, to the fact that, with high speed and with 2 and 3 minutes' pearling, the proportion pearled off is very large and there is only a small quantity left. This suggests that combinations of speed and time should be so chosen that the amount pearled off will not be excessive. It will also be noted that the slopes of the curves are greatest for the high speeds and least for the low speeds, indicating that inaccurate timing would cause greater errors in pearling at high than at low speeds.

It would appear that there is no fundamental reason why different laboratories employing slightly different techniques (regarding speed and time) should not be able to obtain the same relative results, if not the identical values. Since there is apparently a wide latitude in the selection of speed and time, it would seem advisable to select conditions that are readily obtainable. The time interval is no problem, as it can be easily measured by means of a stop watch. The selection of the proper speed resolves itself into the selection of either a standard-speed motor or of driving the pearler through a system of pulleys and belts so that the proper speed is obtained. As was demonstrated in this experiment, a standard-speed motor (1,725 rpm) gives entirely satisfactory results. In view of these considerations, it seems best to recommend a speed of 1,725 rpm, with a pearling time of 1 minute when used with a stop watch and the proper screen as outlined elsewhere in this paper.

Effect of Size of Charge

In the experiment designed to determine the relation between the size of the charge and pearling, Kharkof, Blackhull, and Dawson wheats

TABLE IV

EFFECT OF AMOUNT OF WHEAT USED ON AMOUNT PEARLED OFF

Charge	Wheat of indicated variety pearled off		
	Dawson	Blackhull	Kharkof
g	%	%	%
20	56.7	43.6	35.3
18	60.4	46.2	36.5
16	63.5	48.5	38.7
14	66.1	50.9	42.0
12	69.4	53.9	43.9
10	71.8	57.3	47.6

were pearled under charges of 10, 12, 14, 16, 18, and 20 g. Five replicate determinations were made for each variety with each charge. The averages are presented in Table IV.

These data are plotted graphically in Figure 2. As can be seen from

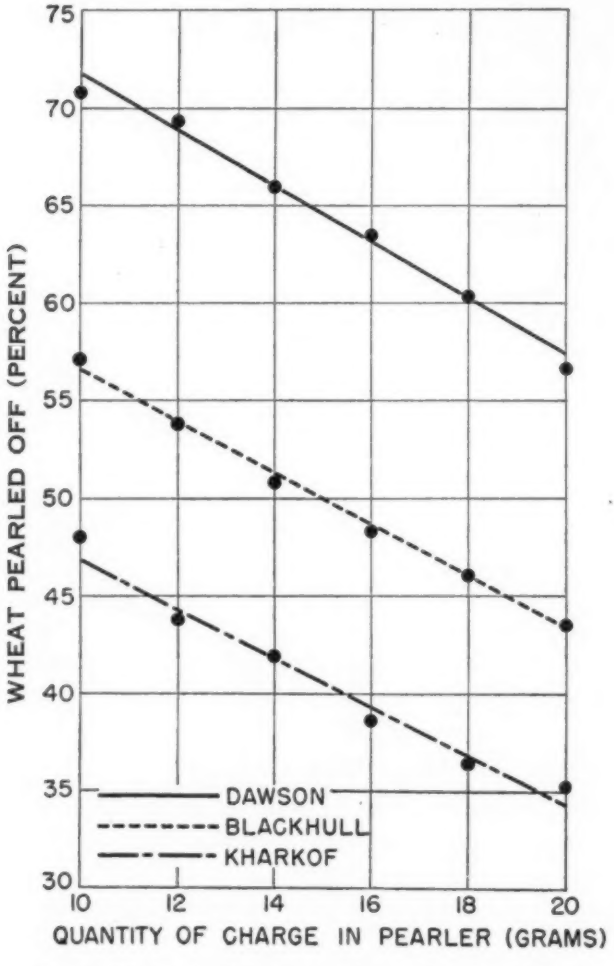

Fig. 2. Effect of size of charge upon amount of wheat pearled off.

this figure the amount pearled off is quite different for each charge, although differences between varieties for any given charge are substantially the same. It would appear, therefore, that a standard procedure should be adopted; otherwise the charge used for any test must be specified.

Effect of Screen

The pearling machine as purchased was equipped with a screen having 8 meshes to the inch. It was discovered that this allowed parts of the wheat kernel to pass through, thereby introducing errors. The machine was then equipped with a 10-mesh screen made of wire 0.041 inch in diameter, Tyler code "Fijor." This is referred to in this paper as the regular screen.

It was questioned whether the revolving stone or the screen did most of the work. Therefore, the screen was removed and replaced with a plain piece of sheet metal. This proved unsatisfactory as there was no place for the ground material to escape and the results were very erratic. A series of $\frac{1}{16}$-inch holes was then drilled in the blank near each edge and it was found that these holes allowed the ground

TABLE V

Effect of Screen on Pearling Results

Screen and time of pearling (minutes)	Wheat of indicated variety pearled off		
	Chiefkan	Blackhull	Dawson
	%	%	%
Regular screen (10-mesh):			
1	35.1	46.7	61.5
2	61.3	77.0	87.5
3	79.1	92.0	—
Blank screen:			
1	8.4	9.4	10.9
2	14.7	18.6	20.7
3	20.4	25.8	30.9

material to escape and the results obtained were fairly accurate. This is the blank referred to in the following experiment.

Samples of Chiefkan, Blackhull, and Dawson wheat were pearled for 1, 2, and 3 minutes with the machine equipped alternately with the regular screen and the blank. These tests were replicated and the averages are tabulated in Table V.

From the data in Table V it is obvious that the greater part of the grinding action is by the screen rather than the stone. This makes it imperative that a new screen be placed in the pearler from time to time, if comparable results are to be obtained over relatively long periods. The fact that some grinding was taking place with the blank screen indicates that part of the work is done by the stone. It, too, should be replaced whenever it appears that it is worn appreciably. While it is practically impossible to determine how much error is being caused by worn screens or stones, it is possible to prevent the error, whatever its magnitude, by merely installing a new screen and stone from time

to time. It is pertinent to note that these varieties are ranked in the same order regardless of which screen is used.

Effect of Moisture Content of Hard Red Winter Wheat

It has been suggested that the moisture content of wheat might have some effect upon pearling results. To determine whether this is the case, six varieties of hard red winter wheat from two experiment stations were used. Each sample was subdivided into nine portions. Some of these were dried by means of warm air until they contained less than the original moisture content; the moisture content of others was increased by exposure to water vapor. As a result of these operations, a series of samples of each variety was obtained containing moisture covering the range of 7 to 15%. After a week these samples were

TABLE VI

EFFECT OF VARYING MOISTURE CONTENT OF WHEAT UPON PERCENTAGE OF WHEAT PEARLED OFF

Variety or station	Wheat (%) of indicated moisture content (%) pearled off									
	7%	8%	9%	10%	11%	12%	13%	14%	15%	Av
Kharkof	32.7	30.0	32.7	30.5	31.0	31.0	32.0	31.8	33.2	31.7
Blackhull	40.2	38.0	40.0	42.2	37.7	37.5	39.2	38.0	38.0	39.0
Tenmarq	31.5	32.5	34.0	32.7	32.3	32.0	30.7	34.0	32.3	32.4
Pawnee	37.2	38.2	37.7	37.0	34.7	36.5	35.5	38.2	37.0	36.9
Comanche	35.5	35.5	36.2	37.2	34.2	36.5	37.7	38.5	37.2	36.5
Chiefkan	37.7	33.2	35.5	34.5	33.0	36.0	36.2	38.0	35.2	35.5
Average	35.8	34.6	36.0	35.7	33.8	34.9	35.2	36.4	35.5	
Hays, Kans.	37.0	35.2	36.5	37.5	33.7	35.2	36.0	37.5	37.3	36.4
Lincoln, Nebr.	34.8	33.8	35.5	34.0	33.5	34.8	35.3	35.3	33.5	34.5

pearled by the basic procedure. The averages of the percentage pearled off are presented in Table VI.

The coefficient of correlation between the moisture content of the wheat and the percentage pearled off was found to be +.029. There was considerable variation in the percentages pearled off. In an effort to learn the reasons for these variations the data were subjected to an analysis of variance. The summary of this analysis is presented in Table VII. It is apparent that the variances in the percentages pearled off were caused by the variety and the station at which the sample was grown and that the moisture content had very little effect. While there is some interaction between the variety and station (the varieties are not equally hard at both stations), there was no interaction between either the station or the variety and the moisture content of the wheat. On the basis of these data it can be assumed that varia-

TABLE VII

ANALYSIS OF VARIANCE OF DATA SHOWN IN TABLE VI

Cause of variance	Degrees of freedom	Mean square	*F*
Variety	5	28.75	26.13**
Station	1	17.10	15.55**
Moisture content	8	1.90	1.73
First-order error	93	1.10	—
Variety × station	5	10.50	16.15**
Variety × moisture	40	.45	.69
Station × moisture	8	.90	1.38
Second-order error	40	.65	—
Total	107	—	—

** Highly significant.

tions in moisture content of the hard red winter wheat within the limits of this study have little or no influence on the percentage pearled off.

Results Obtained by Different Laboratories with Check Samples

To determine the abili ty to check results, four different laboratories[3] collaborated in testing a series of samples. Laboratory B used an old-style pearler, while the other three laboratories used the model 38 pearler. Five varieties of wheat were used for the study. All the samples were submitted under code number without any information as to their nature. The experiment was divided into three parts. (1) The samples numbered 1 to 10 (two of each variety) were to be run by the regular procedure in use at the laboratory where the determination was to be made. (2) The laboratory was to adjust its time of pearling (and speed, if necessary) until it obtained 12.3 g of pearled wheat from a standard sample (so marked) provided for that purpose. (3) Using the time as determined by the standard sample the laboratory was to run the samples numbered from 11 to 20 (two of each variety). A detailed outline of the procedure for running the samples, which was essentially the same as the basic technique described herein, was furnished with the samples. The averaged data obtained in this study are presented in Table VIII.

Two outstanding implications are suggested by these data. First and most important is that all the laboratories, using their own techniques, obtained essentially the same ranking of the samples, even though the weights of pearled wheat varied considerably. The other implication is that by standardizing their technique on the basis of the amount of wheat pearled off of the same sample, all the laboratories not only obtained the same rankings of the varieties but also had nearly

[3] Acknowledgment is made of the cooperation of C. C. Fifield, V. H. Morris, and K. S. Quisenberry, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and Dale Weibel, of the Nebraska Agricultural Experiment Station, in making this collaborative study.

the same over-all range and average weights of pearled wheat. The differences for the latter between laboratories for any one sample were within the limits of experimental error. These facts are further brought out by the coefficients of correlation shown in Table IX.

TABLE VIII

PERCENTAGE OF WHEAT PEARLED OFF IN 4 DIFFERENT LABORATORIES

Variety	Sample No.	Wheat pearled off, under indicated pearling time and speed of stone, at laboratory—			
		A	B	C	D
		1 minute; 1,725 rpm	2 minutes; 1,725 rpm	3¾ minutes; 1,215 rpm	1¼ minutes; 1,725 rpm
REGULAR PROCEDURE					
		%	%	%	%
Chiefkan	3	32.6	24.4	23.5	32.9
	4	32.5	24.5	23.7	32.6
Kharkof	5	33.8	25.3	25.5	34.3
	10	33.7	24.6	25.6	34.5
Blackhull	2	41.3	30.3	29.3	40.7
	6	41.0	30.4	29.7	41.3
Trumbull	1	53.5	37.8	38.7	53.5
	8	53.8	36.7	39.8	53.3
Dawson	7	56.7	38.9	40.6	56.8
	9	56.5	38.7	40.7	56.6
Mean		43.5	31.2	31.7	43.7
Range		24.2	14.5	17.2	24.2
Variety	Sample No.	1 minute; 1,725 rpm	3 minutes; 1,725 rpm	1⅝ minutes; 1,745 rpm	1¼ minutes; 1,725 rpm
PEARLING TIME AND SPEED ADJUSTED					
Chiefkan	15	32.4	33.6	33.7	31.2
	20	32.5	34.1	34.8	32.3
Kharkof	12	33.6	35.9	35.8	33.6
	19	33.7	35.5	35.8	34.8
Blackhull	11	41.3	42.6	42.8	41.8
	16	41.2	43.5	43.4	40.5
Trumbull	14	53.5	53.3	53.2	51.0
	17	53.5	55.0	52.9	52.4
Dawson	13	56.8	54.7	56.1	54.8
	18	56.8	55.6	56.6	57.4
Mean		43.5	44.4	44.5	43.0
Range		24.1	22.0	22.9	26.2

TABLE IX

COEFFICIENT OF CORRELATION AND REGRESSION BETWEEN DATA OBTAINED AT DIFFERENT LABORATORIES AND AT THE SAME LABORATORY BY DIFFERENT TECHNIQUES

Factors correlated	Coefficient of correlation, r	Coefficient of regression, b
Regular technique:		
Between laboratories—		
A and B	+.998	+.600
A and C	.998	.698
A and D	.999	.979
B and C	.990	.850
B and D	.996	1.622
C and C	.995	1.382
Standardized technique:		
Between laboratories—		
A and B	.985	.871
A and C	.988	.874
A and D	.984	.932
B and C	.986	.881
B and D	.999	1.071
C and D	.997	1.070
Between regular and standardized techniques: Laboratory—		
A	.989	1.004
B	.995	1.485
C	.994	1.266
D	.994	.978

These coefficients of correlation and regression are further proof that the laboratories obtained essentially the same results on the samples studied. It appears, therefore, that the technique of different laboratories can be so adjusted as to obtain essentially the same results.

Proposed Standard Technique

The following is a proposed standard technique, based on the experiments reported in this paper and upon laboratory experience with the pearling test over a period of more than three years:

Equipment.

1. Strong-Scott barley pearler, model 38, equipped with a No. 30 grit stone, a 10-mesh screen of wire 0.041 inch in diameter (Tyler code Fijor) and driven at a speed of 1,725 rpm.
2. Stop watch or other timer of equivalent accuracy. (Interval timers of the current-interrupting type have been found unsuitable.)
3. Balance sensitive to 1 cg.
4. Sieve covered with No. 20 wire.

Methods.

1. Each charge (20 g) is weighed from cleaned, unsized wheat that has been thoroughly mixed.
2. The charge is placed in the machine with the stone running at full speed; 60 seconds later the slide outlet is opened; and 10 seconds later the motor is stopped.

3. The pearled wheat is sifted over the 20-wire screen to remove dust and powdered material. The weight of the material remaining on the screen is recorded as the weight of pearled wheat.

Results.

1. Triplicate determinations should be made on each sample. These replicates should be averaged and the results expressed as the percentage of the original sample removed in pearling (percentage pearled off).

Pearling Data for Varieties of Hard Red Winter Wheat in 1938, 1939, and 1940

The relative effects of variety and environment on kernel hardness of the grain as measured by the pearling test are illustrated by data for six varieties of wheat grown at five stations in the southern Great Plains in 1938, 1939, and 1940, as shown in Table X. Since the supply of grain was limited in some cases, only two determinations, instead of three, as recommended, were made of each lot. Otherwise the tech-

TABLE X

EFFECT OF ENVIRONMENT, VARIETY, AND CROP YEAR UPON PERCENTAGE OF WHEAT PEARLED OFF

Variety and crop year	Wheat pearled off at—					
	Amarillo, Tex.	Denton, Tex.	Chillicothe, Tex.	Hays, Kans.	Lincoln, Nebr.	Average
Kharkof:						
1938	33.0	23.5	39.5	30.0	29.0	
1939	36.5	35.0	35.0	34.5	34.5	32.9
1940	34.0	30.0	38.0	30.5	30.5	
Blackhull:						
1938	41.5	38.0	45.5	34.0	42.5	
1939	42.5	41.5	42.0	39.5	39.0	40.7
1940	39.5	41.0	50.0	39.5	34.5	
Tenmarq:						
1938	31.0	32.5	35.0	34.5	30.0	
1939	31.5	37.0	42.5	39.0	37.0	36.0
1940	36.0	42.5	44.0	35.0	33.0	
Pawnee:						
1938	35.5	35.0	35.5	35.5	33.0	
1939	40.5	41.0	41.5	35.0	36.0	36.6
1940	36.5	38.0	38.5	35.0	32.5	
Comanche:						
1938	36.0	37.0	36.5	34.5	37.5	
1939	35.5	36.0	41.5	36.5	31.5	36.3
1940	34.0	35.5	42.5	35.0	34.5	
Chiefkan:						
1938	30.5	31.5	33.5	34.0	31.0	
1939	34.5	29.5	35.0	33.0	31.5	32.7
1940	33.0	31.0	39.5	31.0	31.5	
Average	35.6	35.3	39.8	34.8	33.8	

TABLE XI

ANALYSIS OF VARIANCE OF DATA SHOWN IN TABLE X

Cause of variance	Degrees of freedom	Mean square	*F*
Variety	5	25.90	18.50**
Station	4	18.70	13.36**
Year	2	8.60	6.14**
Error	78	1.40	—
Total	89	—	—

** Highly significant.

nique was as described in the preceding section. These data were subjected to an analysis of variance (Table XI).

The results of this analysis show that the most important influence on kernel hardness is the variety. That is, the kernel hardness is a varietal characteristic but is modified to some extent by the growing conditions as represented by the crop year and the station at which the sample was grown. It will be observed (Table X) that some varieties tended to vary less at the different stations in the same and different years than did other varieties. This tolerance to changing growing conditions may be a desirable characteristic to be considered in developing new wheat varieties. It will be observed, too, that at certain stations there tended to be less difference between the varieties. This may be an indication that under those growing conditions the choice of variety is unimportant as far as it may affect the kernel hardness of the resulting crop.

The results would seem to justify the conclusion that the pearling test measures a physical characteristic that is a varietal function and that it should be useful in classifying and studying varieties of wheat, especially from the plant breeder's viewpoint.

Summary and Conclusions

In a study of various factors thought to affect the pearling test for kernel hardness in wheat, it was found that the amount of wheat pearled off was not materially affected by normal variations in temperature nor by the moisture content of the wheat within the limits studied, *i.e.*, 7 to 15%. Sifting the pearled wheat over a 20-wire screen slightly increased the standard deviation in one case and decreased it in another, although general experience has indicated some improvement in accuracy by sifting. The size of the charge greatly affected the amount of wheat pearled off though not the relative differences between varieties.

By substituting a blank for the regular screen, it was determined that most of the grinding is done by the screen. It appears, therefore,

that both screen and stone should be replaced from time to time if consistent results are to be expected.

By adjusting the time of pearling, it was found that essentially the same results could be secured with speeds of 1,725, 1,520, and 1,300 rpm. In a collaboration study with three other laboratories, involving five varieties of wheat, it was found possible to obtain substantially the same results at all laboratories by adjusting the speed and time of pearling on the basis of the amount of wheat pearled off of a standard sample of wheat supplied for the purpose.

The following standard procedure is recommended. A 20-g charge of cleaned unsized wheat, is pearled for 1 minute at a speed of 1,725 rpm and is sifted over a 20-wire screen in accordance with details previously specified.

In a study of six varieties of hard red winter wheat grown at five stations in the southern Great Plains in each of three years, it was found that kernel hardness, as measured by the pearling test, is determined mostly by variety but also to a considerable extent by the environmental factors of location and year of growth.

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A NOTE ON THE DAMAGE TO WHEAT CAUSED BY THE INDIAN MEAL MOTH[1]

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The necessity for storing large quantities of wheat in bulk for long periods has created new problems on the North American continent. Among these, the control of insect infestation and the grading of grain damaged by insects are important. An opportunity recently arose for examining the effect on wheat quality of the damage caused by the larvae of the Indian meal moth (*Plodia interpunctella* Hbn.). These

[1] Published as Paper No. 62 of the Grain Research Laboratory, and as No. 215 of the Associate Committee on Grain Research.

larvae confine their feeding to the germ portion of the kernel and remove it entirely. As the literature contains no specific data on this matter, it seemed worth while to publish this short note.

Two sets of paired samples, each consisting of degermed kernels and a corresponding lot of normal kernels, were available for study. The kernels were hand-picked from two samples taken from a large elevator annex. Records show that this annex contained One Northern wheat which had remained undisturbed for about two years, and that during the second summer of storage the surface layers suffered considerable damage from a heavy infestation of the Indian meal moth.

Except for the lack of germ ends the kernels appeared quite normal, but the nature of the damage indicated that the yields of mill offals would be reduced. Ward (1943) states "the scutellum and epithelium layers are the centers of B_1 concentration within the grain"; therefore, since these layers are probably removed by the insect along with the other embryo structures, a reduction might also be expected in the thiamine content of the flour. For these reasons special attention was paid to the yields of offals obtained in the experimental milling, and thiamine determinations were made on the flours in addition to making the customary chemical analyses.

As corresponding samples gave similar results for all determinations, only the mean data are reported in Table I. Chemical data and flour absorptions are reported on a 13.5% moisture basis. Yellow pigment (expressed as carotene) was determined by the method of Binnington *et al* (1941), using water-saturated n-butyl alcohol as the solvent. Gassing power, expressed as ml total gas evolved, was determined with sugarless dough (25 g flour and 3% yeast) fermented at 30°C for 6 hours according to the Bailey-Johnson procedure (1924); and thiamine by the A.A.C.C. thiochrome method. Baking quality was determined by the malt-phosphate-bromate procedure as outlined by Aitken and Geddes (1934).

As the proportion of endosperm to offal-producing material is higher in degermed than in normal kernels, the lower yields of mill offals and the higher yield of flour found for the degermed wheat is in accordance with expectation. Otherwise the milling characteristics of the two wheats were essentially the same. In protein content, differences between the wheats, the bran, and the flours were small; but the values for the bran-chips, the shorts, and the feed flour were distinctly higher for the normal kernels. Moreover, the degermed wheat flour was lower in both ash and yellow pigment contents. As the germ is the kernel structure which is high in all of these properties, the lower values for the offals of the insect-damaged grain are a re-

TABLE I

QUALITY CHARACTERISTICS OF NORMAL AND DEGERMED WHEATS

Sample	Normal	Degermed
Bushel weight, lb	63.6	62.2
Milling yields:		
Bran, %	14.6	13.2
Bran-chips, %	3.5	2.8
Shorts, %	4.7	4.3
Feed flour, %	3.2	3.4
Long-patent, %	71.0	73.6
Chemical data:		
Protein, %		
Wheat	13.8	13.4
Bran	15.4	15.2
Bran-chips	18.4	15.5
Shorts	16.6	14.2
Feed flour	14.4	13.1
Long-patent	13.0	13.2
Ash, %	0.48	0.43
Yellow pigment, ppm	2.27	2.18
Gassing power, ml	258	261
Thiamine, μg/g	1.61	0.47
Baking data:		
Absorption, %	60.4	60.6
Loaf volume, cc	770	700
Crumb texture (10)	7.0	6.0
Crumb color (10)	7.5	7.0

flection of the removal of the germ by the insects. Gassing power was almost identical for both flours.

The difference between the thiamine content of the flours is most striking, the values being 1.61 and 0.47 μg per g (243 and 72 International Units per lb) for the normal and degermed samples respectively. This wide spread in thiamine content indicates quite clearly that the scutellum and epithelium—either wholly or in part—were also removed by the insects. It is also apparent from these data that a relatively low percentage of degermed kernels in the mill mixture would lower the thiamine content of the flour appreciably. This might be a matter of some concern to a mill making "Canada Approved Flour" which must have a minimum thiamine content of 2.65 μg/g (400 I. U. per lb).

Turning now to the baking data, we find that the degermed wheat gave a loaf which was definitely inferior to that of the normal wheat in all characteristics; the volume was reduced by almost 9%, and the crumb texture and crumb color were inferior. There were essentially no differences in either absorption or dough-handling properties.

It is difficult to account for the inferior baking quality of the degermed wheat, and no clear-cut explanation can be offered at the

present time. It is known, however, that the kernels were degermed last summer; hence their viability, or germinating power, was destroyed several months ago. This suggests a condition parallel to that found by Swanson (1938) who states: "If the viability is low it (wheat) has suffered some damage (to baking quality), somewhat in proportion to the loss of viability. If the wheat is dead, the damage may be considerable, but the deterioration does not stop when the wheat dies. Hence, how seriously it is damaged depends upon how long it has been dead." This statement must also apply to degermed kernels, which are obviously "dead"; hence the quality of such wheat may be expected to decrease further with continued storage.

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EFFECTS OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. IV. EXPOSURE OF FIVE VARIETIES TO LIGHT RAINS DURING HARVEST [1]

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The 1941 wheat harvest season was moderately dry at Manhattan, Kansas. Five small rains, which fell in the 46 days during which periodic cuttings of wheat were made, totaled less than one inch. Therefore the changes in the physical and other properties observed in wheat samples cut on different days and weeks during the 1941 harvest were the results of exposure to small rains, sunshine, and dews.

The rainfall record at Manhattan during the three months in which

[1] Contribution No. 98, Department of Milling Industry.

the 1941 studies were made is given in Table I, which shows that 3.86 inches of rain fell during the period June 1 to 10. The last 0.76 inch fell during the night before the first cutting was made (June 10). From that time until July 21, when the last cutting was made, only 0.98 inch fell in five small rains.

The first part of the 1941 harvest period was fairly dry in the greater part of the wheat area of Kansas, while the latter part was unusually wet. This gave opportunity to make comparative observations on wheat samples which had been exposed only to slight rains or none at all and those which had been exposed to several rains (Swanson, 1943). The data obtained on those samples showed that the weathered samples, as compared with the nonweathered, had lower test weights and higher mealy percentages, but the flour yields

TABLE I

RAINFALL AT MANHATTAN, KANSAS, JUST BEFORE AND DURING THE 1941 WHEAT HARVEST, AND DURING EXPOSURE IN THE SHOCK

Date	Inches		Date	Inches	
June 1	0.02		July 27	0.72	
6	0.51		30	0.14	
7	0.46		Aug. 11	1.90	
8	1.23		12	0.07	
9	0.88		13	0.06	2.89
10	0.76	3.86[1]	18	0.29	
11	0.14		24	0.20	
13	0.09		26	0.48	
July 2	0.30		Sept. 1	0.16	
3	0.32		2	0.99	
7	0.13	0.98[2]	3	0.06	

[1] Before harvest started.
[2] During entire harvest.

were equally high and the baking values better. The results obtained from the samples harvested at Manhattan where the conditions were comparatively dry are presented in this paper.

Reports of Previous Experiments

A higher percentage of yellow berry, indicating a more mealy condition, was observed in samples cut last in the 1934 harvest; the moisture content of these samples was 13% as compared with the more vitreous condition of the samples cut earlier, when the moisture contents were higher (Swanson, 1936). The soaking of the wheat heads for 10 to 30 minutes had only small effects on test weights. Reported longer soakings of wheat heads in the 1940 experiments progressively decreased the test weights and the decrease was somewhat larger for

the samples dried in the shade than for the samples dried in the sun. Artificial wetting of the grain resulted in greater decrease in test weight than soaking the wheat heads (Swanson, 1941). The lowering of test weight was accompanied by an increase in the mealy condition and a decrease in the percentage of vitreous kernels. That the amounts or degrees of wetting had greater effects than did the duration or number of wettings has also been shown (Swanson, 1943).

General Plan for the 1941 Harvesting Experiment

Five varieties, Early Blackhull, Kanred, Tenmarq, Blackhull, and Chiefkan, all hard winter wheats, were included in the 1941 experiments. Early Blackhull, Kanred, and Tenmarq were cut at various stages of maturity from plots in the variety test field of the Department of Agronomy. The cuttings of each of these varieties were so timed that samples were obtained in the hard dough, hard flinty, and hard bleached stages. Early Blackhull was in the hard dough stage June 10 and Kanred and Tenmarq were in that stage 6 days later. Two small cuttings each of Tenmarq and Chiefkan were obtained from the nursery plots. The first cutting of each of these varieties was made on June 24, at the hard dough stage, the last on July 21, representing maximum exposure. The "Master Plot" of the Kansas Wheat Improvement Association afforded opportunity to cut three small bundles each of Blackhull, Chiefkan, and Tenmarq. Because of the ravages of birds, all these were cut June 21 at the hard dough stage. These bundles were divided into three groups for artificial wetting. Group 1 was not wetted. Group 2 was wetted twice and Group 3, three times. The wetting was done towards evening by immersing the bundles, heads down, in large cans for about 3 hours, after which they were removed from the water. The following morning the bundles were dried in the sun before the next wetting.

Because of the prevailing dry weather, it was thought advisable also to wet once by soaking the bundles of Early Blackhull, Kanred, and Tenmarq cut on July 21. After drying these were placed indoors until threshed.

There was also cut on June 20 from the general field of Tenmarq located near the other plots, such amounts as to build four shocks of 10 bundles each. This wheat was in prime condition for binder harvesting and was not exposed to any wetting by rain. Three of the shocks had two cap bundles each and the fourth was covered with water-proofed canvas. One of the bundle-capped shocks was put in the nursery shed on August 15 after having been exposed to eight rains totaling 3.64 inches. The other three were left outdoors until

September 5, when all were threshed. These were exposed to six more rains or a total of 5.82 inches of moisture.

Procedure in Testing

The test weights were determined by the official method except for the small samples, for which the micro method was used (Swanson, 1942). The grain grading was done by Martin Schuler of the Kansas City office of the Grain Inspection Department, using the figures for test weight as submitted. The internal texture characteristics were observed on the cut kernel sections obtained with a barley cutter and so counted that the figures obtained were in percentages, as in previous experiments (Swanson, 1941). The milling was done on a Buhler experimental mill, with a constant roll setting. The percentages of flour yield were calculated on the weight of samples before they were scoured. Ash determinations were made to check the milling results. The diastatic activity determination gave information on the effects of prolonged exposures to moisture. Since the results of baking tests are influenced by variety as well as by exposure to rain during harvest, only the Tenmarq samples were baked.

Data Obtained

The data, except those for baking, are given in Tables II and III. The figures for rainfall represent the total amount of rain (calculated from the data in Table I) to which the samples had been exposed between the hard dough stage and cutting. The 0.23 inch and the 0.62 inch each fell in two rains. The 0.75 inch fell in three and the total of 0.98 inch fell in five light rains. Where no figures are given, the sample had received no rain from the hard dough stage until cut. The comparatively large rains each day from June 6 to 10 seemed to have had no noticeable effect on the physical texture.

Test weight: From the time of the first cutting until July 14, the decrease in test weight was 4.2 pounds for Early Blackhull, 3.4 pounds for Kanred, and 2.6 pounds for Tenmarq. A much larger decrease than this would be obtained for Tenmarq if the 61.8 pounds had been used as the first test weight, but as this is so much larger than the other test weights obtained in connection with the shocks it seems that the 61.6-pound test weight sample was from wheat that was more plump than that from other parts of the field. This sample had about 2% lower protein than the others. The Tenmarq sample cut in the Agronomy Nursery, which had been exposed to 0.84 inch, decreased 4.3 pounds in test weight. The Chiefkan sample from the nursery showed no decrease. This may be due partly to field error, since the

TABLE II

EFFECT OF TIME OF HARVESTING AND EXPOSURE ON TEST WEIGHT, GRADE, TEXTURE, MILLING, AND DIASTATIC ACTIVITY

Serial No.	Variety and condition at harvest	Rainfall	Test weight		Grade	Texture counts, no. in 100			Flour yield	Flour ash	Diastatic activity
			Cleaned	Scoured		Vitreous	Semi-vit.	Mealy			
		in	*lbs*	*lbs*		%	%	%	%	%	*mg*
	From Agronomy Farm										
1035	Early Blackhull, cut June 10, hard dough		64.0	66.4	1 DHW	93	3	4	69.9	.395	165
1036	Early Blackhull, cut June 19, hard flinty	0.23	63.2	66.0	1 DHW	98	2	0	73.0	.416	122
1037	Early Blackhull, cut July 14, hard bleached	0.98	59.8	62.7	2 DHW	22	33	45	70.9	.385	108
1038	Early Blackhull, cut July 21, hard bleached wetted	0.98	58.6	61.5	2 HW	8	28	64	70.0	.390	109
1039	Kanred, cut June 16, soft dough border	—	57.5	61.3	3 DHW	100	0	0	70.1	.491	141
1040	Kanred, cut June 16, hard dough	—	58.7	61.9	2 DHW	100	0	0	72.2	.468	128
1041	Kanred, cut June 24, hard flinty	—	58.6	62.0	2 HW	96	2	2	71.6	.470	130
1042	Kanred, cut July 14, hard bleached	0.75	55.5	60.1	4 DHW	74	23	3	74.0	.446	131
1043	Kanred, cut July 21, hard bleached wetted	0.75	55.3	59.7	4 DHW	62	31	7	73.3	.452	136
1047	Tenmarq, cut June 16, hard dough	—	61.6	63.7	1 DHW	79	7	14	73.8	.503	151
1048	Tenmarq, cut June 24, hard flinty	—	57.8	61.6	3 DHW	98	2	0	72.9	.515	150
1049	Tenmarq, cut July 3, hard bleached	0.62	56.6	60.5	3 DHW	30	58	12	76.0	.496	162
1050	Tenmarq, cut July 14, hard bleached	0.75	55.3	59.4	4 DHW	28	66	6	72.4	.510	154
1051	Tenmarq, cut July 21, hard bleached wetted	0.75	54.5	58.4	4 DHW	21	72	7	73.5	.503	124
1052	Tenmarq, threshed Aug. 15, shock bundle covered	3.64	57.9	61.6	3 DHW	98	1	1	72.4	.530	154
1053	Tenmarq, threshed Sept. 5, shock bundle covered	5.82	56.0	59.9	3 DHW	93	3	4	75.5	.530	285
1054	Tenmarq, threshed Sept. 5, shock bundle covered	5.82	56.5	60.0	3 DHW	91	6	3	74.0	.482	430
1055	Tenmarq, threshed Sept. 5, shock canvas covered	5.82	57.9	60.9	3 DHW	92	6	2	71.9	.498	260

TABLE III

EFFECT OF TIME ON HARVESTING AND WETTING ON TEST WEIGHT, GRADE, AND TEXTURE

Serial No.	Variety	Description	Rain	Test weight		Grade	Texture counts, no. in 100		
				Cleaned	Scoured		Vitreous	Semivit.	Mealy
			in	*lbs*	*lbs*		%	%	%
		From Ag. Nursery							
1064	Chiefkan	Cut June 24	—	59.8	—	2 DHW	98	1	1
1065	Chiefkan	Cut July 21	0.84	59.9	—	2 DHW	85	12	3
1066	Tenmarq	Cut June 24	—	58.6	62.3	2 DHW	99	1	0
1067	Tenmarq	Cut July 21	0.84	54.3	59.0	4 HW	48	45	7
		From Master Plot							
1056	Tenmarq	Cut June 21, no treatment, hard dough	—	60.1	62.9	1 DHW	99	1	0
1057	Tenmarq	Cut June 21, wetted June 26, 28	—	56.9	60.5	3 DHW	74	17	9
1058	Tenmarq	Cut June 21, wetted June 26, 28, 30	—	56.1	60.2	3 DHW	74	18	8
1059	Chiefkan	Cut June 23, no treatment, hard dough	—	62.5	—	1 DHW	97	1	2
1060	Chiefkan	Cut June 23, wetted June 30, July 5	—	60.0	—	1 DHW	88	9	3
1061	Blackhull	Cut June 21, no treatment, hard dough	—	61.4	64.3	1 DHW	91	6	3
1062	Blackhull	Cut June 21, wetted June 26, 28	—	58.3	—	2 DHW	36	47	17
1063	Blackhull	Cut June 21, wetted June 26, 28, 30	—	58.7	—	2 DHW	20	58	22

last sample was cut some distance from the location of the first sample. Chiefkan, however, in several experiments has shown superior resistance to exposure.

Effect of artificial wetting: There was probably only a little decrease in test weight due to exposure in the field of Early Blackhull, Kanred, and Tenmarq during the period July 14 to 21, since no rain fell and hence the decreases of 1.2 pounds for Early Blackhull, 0.2 for Kanred, and 0.9 for Tenmarq were the result of wetting by one soaking of the bundles in large cans for about three hours.

The samples cut from the "Master Plot" showed the following decreases in test weight from the wetting treatment: Tenmarq, 3.2 pounds from the two wettings and 4.0 from the three wettings; Chiefkan, 2.5 pounds from the two wettings; Blackhull, 3.1 pounds from two wettings and 2.7 from three wettings. Since Blackhull was cut in several locations, the smaller decrease from three wettings as compared with two may be accounted for by differences in the samples.

The decreases in test weight from exposure in shocks were much less than that which occurred in the field. The cap bundles were tied on and afforded good protection. It was observed that the straw, even in the bundle-capped shocks, had changed comparatively little. This is in contrast to the large changes which occurred in 1940 as a result of exposure to 5.16 inches of rain (Swanson, 1941), but those shocks were larger and less well protected.

Effect of scouring on test weight: The data in Tables II and III show that all the test weights obtained on the scoured samples were larger than on the unscoured, indicating that the condition of the outside layers of the bran coat has a considerable influence on test weight. The looseness of the outside bran coat, which helps to decrease the test weight, increased with the length of exposure. Thus the increase in pounds of test weight after scouring the first and last samples were: Early Blackhull 2.4 and 2.9; Kanred 3.2 and 4.4; Tenmarq 2.7 and 4.0. The decreases in test weight due to exposure follow the same general trend for the scoured samples as for the unscoured and show that besides the loosening of the bran coat, the internal swelling is also a factor. That this swelling can take place without the soaking due to heavy rains is indicated.

Wheat grades: A comparison of the grades with test weights shows that the test weights were the main determining factors in grading the samples, although other factors such as percentages of vitreousness and total damage were also included in the grading.

Internal texture: The data on texture counts in Tables II and III indicate the internal characteristics of the wheat kernels. A kernel

having entire absence of mealiness in the cut section is designated as vitreous. When the whole surface has a white, floury aspect, the designation is mealy. Between these extremes there are many variations which were all counted as semivitreous. These may tend more toward either the vitreous or the mealy, the trend usually being toward the larger figure. Thus, when the percentages of vitreous kernels are comparatively large, the semivitreous kernels approach the vitreous and likewise when the percentages for the mealy kernels are comparatively large, the semivitreous are more toward the mealy.

The data in Tables II and III show that the vitreous condition of the kernels decreased and the mealy condition increased with the duration of exposure in the field. Of the three varieties harvested on the Agronomy farm, Early Blackhull underwent the greatest change, Kanred the least, with Tenmarq intermediate. In the nursery plots Chiefkan showed only half as much change as Tenmarq.

The wetting by soaking the bundles obtained at the last cutting of Early Blackhull, Kanred, and Tenmarq on July 21 made a notable further decrease in the vitreous condition and increase in the mealy. The wetting treatments of the samples from the "Master Plot," Table III, produced less change in Tenmarq than in Blackhull but had comparatively little effect on Chiefkan. A comparison of the amounts of maltose obtained shows no correlation with the progress of weathering in the field, but the amounts of maltose obtained from Tenmarq were notably higher than from Early Blackhull and Kanred. This indicates a varietal effect as was shown previously by Swanson (1935), who also presented data that showed no increase in the diastatic activity until the moisture content of the wheat kernels was increased to 27% or 30%. The amounts of maltose obtained from the wheat in the shocks showed a distinct increase in diastatic activity due to exposure. This result was also found in a previous experiment (Swanson, 1941).

The 260 mg. of maltose obtained from the canvas-covered shock in comparison with the 154 mg. obtained from the shocks covered with bundles, as well as similar amounts obtained from the Tenmarq samples which had not received any rain, indicate that increase in diastatic activity may occur in the absence of distinct wetting. These results from the 1941 season agree with those obtained in 1940 (Swanson, 1941). In 1940, 233 mg. of maltose was obtained from the canvas-covered shock in comparison with 135 mg. from the check or unwetted sample. No explanation for this is apparent.

Baking tests: The results of the baking tests as given in Table IV were all from Tenmarq and hence variation between varieties was

eliminated. The largest loaf volumes with comparably good crumb textures were obtained from the samples cut on July 14 and 21 and hence had the most exposure. All the other loaf volumes did not show any definite trends that could be correlated with the harvest treatments. The smallest loaf volume from the sample cut June 16 was clearly due to about 2% lower protein content. Table II shows that this sample had a test weight of 61.6 and graded 1 DHW. All the other samples graded 3 and 4 DHW or HW. The diastatic activity seemed to have had no distinct influence on the baking results.

TABLE IV[1]

BAKING RESULTS ON TENMARQ WHEAT EXPOSED TO WETTING

Serial No.	Description	Flour protein	Grade	Baking		
				Loaf vol.	Texture	Crumb color
		%				
	Treatment during harvest					
1047	Cut June 16, hard dough	11.4	1 DHW	793	80-0	83 c-y
1048	Cut June 24, hard flinty	13.6	3 DHW	938	87-0	85 c-y
1049	Cut July 3, hard bleached	13.3	3 DHW	940	80-0	83 c-y
1050	Cut July 14, hard bleached	13.9	4 DHW	1020	80-0	84 c-y
1051	Cut July 21, hard bleached, wetted	13.9	4 DHW	1007	88-0	87 c-y
	Shocks					
1052	Bundle covered	13.7	3 DHW	985	84-0	83 c-y
1053	Bundle covered	13.0	3 DHW	933	84-0	83 c-y
1054	Bundle covered	13.2	3 DHW	988	82-0	83 c-y
1055	Canvas covered	13.3	3 DHW	930	87-0	85 c-y

[1] Credit is due Mr. John A. Johnson for doing this baking.

Discussion

The data presented show that prolonged exposure to small rains, sunshine, and dew in the field after the wheat had reached the hard dough stage markedly lowered the test weight with consequent reduction in wheat grade. The data also show that the vitreous characteristic was greatly decreased, with consequent increase in the mealy condition of the endosperm. As in the previous investigations (Swanson, 1941, 1943, 1943a), neither the percentage of flour yield nor the baking value was lowered.

Because of the smallness of the rains there could have been no such entrance of water into the kernels exposed in the field as takes place when wheat is soaked in water. In a previous investigation (Swanson, 1936), it was shown that soaking wheat heads for 30 minutes and then drying in the sun decreased the test weights and increased the percentage of yellow berry less than when the drying was done in

the shade. The soaking for 10 minutes had less effect than soaking for 30 minutes but for both periods the number of wettings had cumulative effects. The prolonging of the wet condition was a factor as well as the amounts and the number of times wetted. Because of the small rainfall in 1941, no such swelling could have taken place as when wheat kernels are soaked in water for a few hours. Yet there was a reduction in test weight and changes in internal texture similar to those that occurred when wheat was soaked or when the moisture was increased to 26% or more.

It may be assumed that these changes were due to the absorption or adsorption of water into the interior of the kernels. Since the amounts of water were so small that its entrance into the kernels could not have taken place by such movements as are designated as capillarity, the question arises: How does the water enter when insufficient for such movement? The answer to this question rests on general principles of water movement, in both liquid and vapor phases, and on the forces that govern absorption and adsorption.

Tempering water enters into the wheat kernel in much the same manner as the water from small rains, which probably increase the moisture content to about 16% or a little more. When dry wheat is tempered to about 16% moisture for milling, water is first absorbed into the bran layers, from which it is distributed throughout the endosperm. The distribution does not take place by capillary movements because of the firm adsorption of the small amounts of water.

Immediately after dry wheat is tempered for milling, it both feels and appears wet and capillary movements of water can take place in the outer bran layers. In about half an hour or even less the water seems to have been absorbed to such an extent that the wheat feels only slightly damp. In this condition the water films are too thin for capillary movements. That the water has not been distributed throughout the entire endosperm is known from the fact that in tempering practice several hours are required for this distribution to take place.

That wheat kernels have pore spaces among the starch granules and intertwined protein material is known from the fact that the specific gravity of the whole kernel is less than that calculated from the specific gravities of the various substances. These pores vary in shape, configuration, and in size from microscopic to submicroscopic. Even the latter are large in relation to the diameter of the water molecule, which is estimated to be 0.2 mμ, and hence many layers of water molecules can be adsorbed on the materials, mostly starch and protein, which form the walls of these pores.

Just after the tempering water has been absorbed the layers of water molecules are most numerous in the outer bran portions. The adsorbed layer or layers of water molecules next to the surfaces of the solids are held by the force of adhesion. The layers superimposed on these are held partly by the forces that emanate from the solid and partly by the force of cohesion among the water molecules. Since the forces from the solid surfaces decrease exponentially, the outer layers of molecules are held less and less firmly. These outer layers have then more vapor pressure or energy to escape than those nearer the surface of the solids. Hence the molecules in the outer layers will evaporate or assume the gaseous state. Because of their freedom in the gaseous condition they can penetrate further into the pores of the kernel and become adsorbed where the layers of water molecules are less thick. In this way the thicker outer layers of adsorbed water molecules lose water while the layers farther in gain. A continuance of this process will eventually distribute the water evenly throughout the whole interior of the kernel.

The water entering into the interior of wheat kernels in the manner described forces the compact structures of the starch and other substances apart. The swelling of the wheat kernels that takes place from the entrance of water molecules in the vapor state is analogous to what happens when dry wood is exposed to a damp atmosphere.

The repeated wettings that resulted from the light rains and the drying between wettings caused the disarrangement of the original compact interior structure of the endosperm and changed it from a vitreous to a mealy structure. The dews at night probably were a contributing factor.

Conclusions

Data have been presented that show the effects of exposure of five wheat varieties to several small rains during the harvest season of 1941. Effects of wetting by soaking wheat in the straw were also observed.

Exposure to small rains totaling less than one inch decreased the test weight enough to depress the grade from No. 1 to No. 3 and No. 4. The artificial wetting had similar effects. The interior of the endosperm changed from a predominantly vitreous to a predominantly mealy condition.

The lowering of the test weights and the increase in the mealy condition had no effect on the flour yield or on the baking value.

Of the several varieties observed, Early Blackhull and Blackhull showed the greatest change from the vitreous to the mealy condition

of the endosperm. Chiefkan was the least affected, while Kanred and Tenmarq were intermediate.

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THE EXPERIMENTAL ERROR OF THE THIOCHROME METHOD FOR THIAMINE ASSAY[1]

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The expanding manufacture of enriched flours makes it increasingly important to know the experimental error of the assay methods for the added compounds. The intralaboratory error limits a mill's ability to detect variations in its own product, and the interlaboratory error affects the uniformity with which different mills, each operating its own laboratory, can meet standard specifications. The thiochrome method (Cereal Laboratory Methods, 1941), widely employed for the determination of thiamine, is now sufficiently standardized to merit detailed examination. Two studies of its precision have already been reported: the first (Lundie and Robertson, 1941) involved only two laboratories and dealt with data obtained by visual measurement, which probably would yield less precise results than the more common photoelectric methods, while the second (Frey and Hennessy, 1942) reported only interlaboratory errors, and not all collaborators employed the same method. More comprehensive data were recently made available to the present authors, and the analyses of these add useful information to that already published.

These data consist of two series: (1) duplicate values for 68 samples (whole wheat and rye; wheat and rye flours) obtained by the same method in five laboratories of the Products Control Department,

[1] Paper No. 2097, Scientific Journal Series, Minnesota Agricultural Experiment Station.
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General Mills, Inc., and (2) duplicate values for 514 samples of wheat kernels, glumes, and stems, determined by the same method in the Division of Agricultural Biochemistry, University of Minnesota (previously published in part by Geddes and Levine, 1942).

Descriptions of samples of the first series and the errors of analysis are shown in Table I. The errors are given in both absolute (standard error) and relative (coefficient of variability) terms within laboratories and within samples. The "within laboratories," or duplicate, errors represent average performance of all five laboratories. The "within samples" error includes variation between laboratories, duplicate error, and the error resulting from the tendency of the various laboratories to obtain relatively different results with different samples (interaction of

TABLE I

ERRORS OF THIOCHROME METHOD BASED ON COLLABORATIVE ANALYSES IN FIVE LABORATORIES

Group	Nature of flours	Number samples	Thiamine range (Means all laboratories)	Group mean	Standard error of single determination		Coefficient of variability	
					Within labs.	Within samples	Within labs.	Within samples
			mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	%	%
I	Patent	10	0.16–0.38	0.28	0.022	0.033	8.1	12.1
II	Long patent; st. grade	10	0.46–0.78	0.57	0.030	0.046	5.3	8.0
III	White rye; durum	5	0.82–0.99	0.94	0.037	0.065	4.0	6.9
IV	Enr. patent; 1st clear	19	1.26–1.79	1.54	0.054	0.088	3.5	5.7
V	Enr. patent; whole wheat	16	1.84–2.63	2.12	0.066	0.116	3.1	5.5
VI	Clear; dark rye	3	3.03–3.28	3.14	0.076	0.233	2.4	7.4
VII	2d clear; red dog	5	7.27–9.28	8.53	0.218	0.426	2.6	5.0
All		68	0.16–9.28	1.89	0.077	0.169	4.1	8.9

laboratories by samples). Both errors increase with increasing thiamine content. The correlation between thiamine level and difference between duplicates was found to be + 0.88 (value of r at 5% level = 0.23). Accordingly, these data have been segregated into seven classes with samples grouped on the basis of thiamine content. Although the absolute error, both within laboratories and within samples, increases markedly with increasing thiamine content, the change is not strictly proportional. Hence the coefficient of variability (standard error as per cent of the mean) shows a distinct downward trend with increase in thiamine. Because the "within samples" error includes two sources of variation in addition to the duplicate error, it is appreciably larger than the "within laboratories" value in all groups. Nevertheless, the agreement between laboratories, as shown in Table II, is good.

TABLE II

MEAN THIAMINE VALUES AND ERROR BETWEEN LABORATORIES

Class	Number samples	Mean thiamine content for laboratories						Standard error [1] between laboratories
		A	B	C	D	E	All labs.	
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
I	10	0.27	0.27	0.28	0.29	0.27	0.28	0.030
II	10	0.58	0.55	0.57	0.58	0.57	0.57	0.042
III	5	0.95	0.90	0.99	0.94	0.90	0.94	0.062
IV	19	1.52	1.49	1.54	1.58	1.58	1.54	0.084
V	16	2.13	2.14	2.10	2.16	2.05	2.12	0.112
VI	3	3.37	3.15	3.14	2.90	3.13	3.14	0.240
VII	5	8.91	8.73	8.21	8.44	8.39	8.53	0.418
All	68	1.92	1.89	1.86	1.89	1.87	1.89	0.168

[1] Apply to individual sample means for each laboratory.

The data of the second series gave duplicate errors of 0.022 and 0.023 mg/lb for stems and glumes respectively, each of which had an average thiamine content of approximately 0.73 mg/lb. The duplicate error for wheat kernels was 0.043 mg/lb at a mean thiamine level of 2.55 mg/lb. These standard errors are somewhat lower than those of the first series for similar thiamine levels. In the second series, a significant positive correlation was also obtained between thiamine content and difference between duplicates.

The present study shows a greater precision of thiochrome results than is reflected in previous papers. Where the values can be compared, the intralaboratory error of these data is about half of that previously reported, while the interlaboratory error has been reduced even more. Such increase in precision is believed to be the natural result of better standardization of the method and greater familiarity with the technique.

If two samples of enriched flour are analyzed in duplicate in the same laboratory, the difference in the mean values must be at least 0.1 mg/lb to be significant. This follows from the standard errors shown in Table I, assuming the significant difference to be twice the standard error. If the analyses are made in two laboratories, the difference would have to exceed 0.2 mg/lb. We believe that this precision is satisfactory for a method as complex as the thiochrome technique, although it is not impossible that additional refinements will result in further reduction in errors.

Summary

Duplicate analyses of 68 samples of wheat, rye, and wheat and rye flours in five laboratories; and of 514 samples of wheat kernels, glumes,

and stems in another laboratory show that absolute errors of the thiochrome method increase with increasing thiamine content, but not in strict proportion. Such errors tend to be largest, on a percentage basis, with low thiamine samples and to diminish with increasing thiamine content.

At the thiamine level for enriched flour, the means for duplicate analyses must differ by more than 0.1 mg/lb to be significant where the analyses are made in one laboratory; and by more than 0.2 mg/lb where the analyses are made in different laboratories.

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A RAPID METHOD FOR THE DETERMINATION OF THIAMINE IN WHEAT AND FLOUR [1]

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During the last two years the production of flours rich in thiamine has assumed considerable commercial importance. Large quantities of flour enriched with the synthetic vitamin to specified levels are now being milled in the United States and Canada, while substantial amounts of long extraction flours are also being produced for sale under guarantees with respect to thiamine content. As a result of these recent developments, a definite need has arisen for a rapid method for the determination of thiamine in flour, especially for use in the control of milling operations. A single determination by the thiochrome method, as described in Cereal Laboratory Methods, requires about three hours while other published modifications of the procedure take even longer. At least during the period needed for a single determi-

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nation in a control laboratory, flour may be milled which fails to meet the requirements with regard to thiamine.

Many laboratories have simplified and shortened the regular thiochrome method in order to increase the value of their results for mill control purposes, but as yet very little information is generally available as to the nature of the modifications which have been adopted or their effects upon the results obtained. The greatest simplification was proposed by Andrews and Nordgren (1941) who suggested shaking the sample with 25% potassium chloride solution in dilute acetic acid and determining the thiamine in the filtered extract by oxidation followed by the usual extraction with isobutanol. Andrews and Nordgren found this method to be unsatisfactory for bread since enzyme treatment is required to hydrolyze cocarboxylase, but in the case of milled products it gave results in fairly close agreement with those obtained by the regular thiochrome method. They believed that the rapid method would prove of special value in assaying enriched flours for thiamine. Nicholls *et al* (1942) followed a similar procedure except that they extracted their flour samples with dilute hydrochloric acid.

Late in 1941 Canadian mills were faced with the problem of producing long extraction flour of the best possible grade, having a thiamine content of not less than 1.2 mg per pound. To meet the pressing need thus created for a rapid method of thiamine assay suitable for mill control, it was decided to investigate the method proposed by Andrews and Nordgren with the object of determining (1) whether the method was capable of giving reproducible results; and (2) whether the results it yielded, when applied to a variety of wheat products, were in agreement with those obtained by our regular thiochrome procedure. The purpose of the present paper is to report the results of these inquiries. Some data on collaborative samples have also been included as a means of providing information as to the relationship between our thiamine results and those obtained elsewhere. This seemed desirable in view of the wide variations in the results of assays carried out in different laboratories on portions of the same sample.

Experimental

Development of rapid method. After some experience had been gained with the method described by Andrews and Nordgren, the following modified procedure was adopted for further investigation:

Apparatus:

(1) Fluorophotometer.
(2) Centrifuge and 50 ml centrifuge tubes, preferably without lip.
(3) Boiling tubes.

(4) Reaction vessels as described by D. J. Hennessy (1941).
(5) Automatic pipette (16 ml) for isobutanol.

Reagents:

(1) Potassium chloride solution. Dissolve 250 grams in 2% acetic acid and make up to 1 liter.
(2) Sodium hydroxide solution 15%.
(3) Potassium ferricyanide solution 3% (made up fresh each week).
(4) Oxidizing reagent: 1 ml of 3% potassium ferricyanide solution diluted to 100 ml with 15% sodium hydroxide (made up fresh each day).
(5) Isobutanol; re-distilled from all-glass apparatus.
(6) Anhydrous sodium sulphate.
(7) Quinine sulphate solution. Dissolve 0.108 g of Quinine sulphate (U.S.P.) in 0.1*N* sulphuric acid and make up to 1 liter. Dilute 1 to 400 for working standard.
(8) Standard thiamine solution. Weigh out 0.1 g of pure thiamine hydrochloride, previously dried over P_2O_5, and make up to 1 liter with water. Pipette 10 ml of this solution into a 1-liter flask and make up to volume with 0.1*N* hydrochloric acid.

Method:

Place 1 g of sample (or an amount containing 3–5 μg of thiamine in the case of mill feeds and low grade flour) in a 50-ml centrifuge tube and add 20 ml of potassium chloride solution. Break up any lumps by means of a glass rod. Place the centri-

TABLE I

EFFECT OF TIME AND TEMPERATURE ON THIAMINE EXTRACTION

Material	Extraction time	Thiamine		
		26° C	50° C	70° C
	min	*μg/g*	*μg/g*	*μg/g*
Patent flour (Ash 0.37%)	10	0.80	0.79	0.83
	20	0.79	0.82	0.80
	30	0.79	0.83	0.80
Long extraction flour (Ash 0.60%)	10	2.03	2.05	2.10
	20	2.11	2.07	2.16
	30	2.07	2.09	2.22
Second clear flour (Ash 1.20%)	10	5.65	5.72	6.13
	20	5.51	5.89	6.07
	30	5.65	6.07	6.20
Whole wheat flour	10	4.48	4.50	4.57
	20	4.54	4.64	4.75
	30	4.40	4.76	4.90
	60	4.63	4.82	4.93
Bran	10	7.07	7.10	7.66
	20	7.07	7.27	7.65
	30	6.96	7.31	7.89
Shorts	10	16.77	17.63	17.34
	20	16.64	17.73	17.61
	30	17.00	17.90	18.17
	60	—	17.89	18.23
Averages	10	6.13	6.30	6.44
	20	6.11	6.40	6.50
	30	6.15	6.50	6.70

fuge tube in a water bath at 70° C, cover with a boiling tube to prevent evaporation, and leave for 30 minutes. At the end of this time, stir the mixture and break up any lumps that have formed, and then centrifuge at about 2500 rpm for 5 minutes.

Transfer a 5-ml aliquot of the supernatant solution to a reaction vessel. To this add rapidly 3 ml of the oxidizing reagent, followed exactly 1 minute later by 16 ml of isobutanol. Shake for 1½ minutes and centrifuge at low speed for 45 seconds. Run off the aqueous layer, add approximately 1.5 g of anhydrous sodium sulphate and shake for 20 seconds. Pour the clear isobutanol extract into a cuvette and determine the fluorescence.

Adjustment of the fluorophotometer against quinine sulphate solution, standardization with pure thiamine, and the blank determinations, are all carried out as in the regular procedure. This procedure is essentially the same as that outlined in Cereal Laboratory Methods (4th ed. 1941) except that the sodium hydroxide and potassium ferricyanide solutions are combined before use.

Temperatures of extraction both higher and lower than 70° C were tried. Above 70° C increased gelatinization of starch made it more difficult to obtain fairly clear solutions on centrifuging; at room temperature, extraction was found to be incomplete in 30 minutes in the case of bran, shorts, and lower grade flours. Shaking frequently by hand during extraction at 70° C had no appreciable effect on the results.

TABLE II

THE EFFECT OF VARIATIONS IN FERRICYANIDE CONCENTRATION ON FLUOROPHOTOMETER READINGS FOR THE STANDARD THIAMINE SOLUTION AND FOR CERTAIN RAPID METHOD EXTRACTS

Extract	Milligrams ferricyanide						
	0.025	0.05	0.1	0.3	0.6	0.9	1.8
	Mean galvanometer deflection (Coleman) [1]						
Standard thiamine	1.0	73.0	74.0[2]	71.0	68.0	68.0	63.0
Enriched flour	1.0	11.0	87.0	88.5[2]	84.5	80.5	—
Clear flour	2.0	9.5	34.0	96.0[2]	96.0[2]	88.0	75.0
Whole wheat flour	3.0	9.5	33.0	83.0[2]	80.5	73.0	67.5
Shorts (1)	2.5	4.0	15.0	80.0	83.0[2]	73.5	64.5
Shorts (2)	2.5	4.0	13.0	75.0[2]	72.0	70.0	63.5
Bran	—	4.5	10.75	61.0	72.0[2]	68.0	58.0

[1] The blank is subtracted in all cases.
[2] Maximum reading.

The effects of time and temperature of extraction are shown in Table I. The results reported are the averages of duplicate determinations.

Several workers have claimed that the excess of ferricyanide used in the thiochrome method causes fictitiously high values. Excess of ferricyanide reduces the galvanometer readings, and since the excess will vary according to the amount of reducing impurities in the thiamine solution taken for oxidation, greater destruction of thiochrome is to be expected in some cases than in others. The greatest destruction is likely to occur in the case of the standard. It is on that account that the method is suspected of giving high results, the magnitude of the error depending, however, upon the material analyzed.

If this is a cause of error when the thiamine extract is purified by zeolite, a far greater effect might be expected when the extract contains relatively large quantities of organic matter as occurs in the rapid method just described. Several oxidations were therefore carried out on each of a number of extracts using different concentrations of ferricyanide. These amounts of ferricyanide were also used to oxidize the standard thiamine solution. The same quantity of sodium hydroxide (0.45 g) was used throughout. Fluorophotometer readings thus obtained are reported in Table II.

It will be observed that in these experiments the standard gave its maximum fluorescence with 0.1 mg of ferricyanide while some samples showed maximum

fluorescence with 0.3 mg and others with 0.6 mg. The thiamine values calculated on the basis of the maximum fluorescence readings for standard and samples, and the values obtained by oxidizing the standard and the samples with 0.6 mg, 0.9 mg, and 1.8 mg of ferricyanide are given in Table III.

It would seem logical to adjust the quantity of ferricyanide to secure the maximum fluorescence at each oxidation, though even if that were done it would still be necessary to assume that, in all cases, the amounts of thiochrome finally extracted by the isobutanol bore a constant relationship to the thiamine in the extracts. To select the ferricyanide level needed to produce maximum fluorescence would require at least three, and sometimes four or five, oxidations on each extract. Because of the additional work involved in such a procedure, we followed the customary practice of using a fixed amount of ferricyanide.

The quantity of ferricyanide used in all our determinations, whether by the rapid or the regular method, was 0.9 mg. This was more than sufficient to produce the maximum fluorescence in all samples tested [1] and was nine times the amount needed to give the greatest fluorescence in the standard. However, the destruction of thiochrome which occurred when 0.9 mg of ferricyanide was used was of about the same order in all the cases studied, including the standard. As is shown in Table III, the thiamine values obtained with this quantity of ferricyanide were practically the

TABLE III

THIAMINE VALUES OBTAINED BY USING DIFFERENT FERRICYANIDE LEVELS

Extract	Milligrams ferricyanide used for standard and samples			
	Optimum [1]	0.6	0.9	1.8
	μg/g	μg/g	μg/g	μg/g
Enriched flour	4.78	4.97	4.73	—
Clear flour	5.18	5.65	5.18	4.76
Whole wheat flour	4.49	4.74	4.30	4.29
Shorts (1)	14.99	16.27	14.41	13.65
Shorts (2)	13.51	14.11	13.73	13.44
Bran	7.79	8.47	8.00	7.37
Mean (omitting enriched flour)	9.19	9.85	9.12	8.66

[1] Quantity of ferricyanide required to give maximum fluorescence readings for standard and samples.

same as those obtained when the ferricyanide was adjusted to give the greatest fluorescence readings. Oxidation with 0.6 mg of ferricyanide gave higher thiamine values, for at that level some of the extracts displayed their maximum fluorescence, while in the remainder, the effects of excess of ferricyanide were smaller than in the case of the standard. Oxidation with 1.8 mg of ferricyanide gave lower thiamine values. Contrary to what might be expected, increments in the excess of ferricyanide reduced the fluorescence of the standard to a lesser degree than they did the fluorescence readings of the extracts. The presence of substances extracted from the samples apparently caused some increase in the effect of excess ferricyanide on the fluorescence readings.

In the few instances we have studied, thiamine solutions eluted from zeolite reacted like the standard to excess ferricyanide. The same thiamine values were obtained whether 0.2 or 0.9 mg of ferricyanide, or some amount between these limits, was used.

The above data and discussion are inserted here to explain our use of 0.9 mg of ferricyanide for oxidation. It is realized that all samples may not behave in the same way as those we have studied and that the use of a fixed amount of oxidant may cause erroneous results in some instances. We do not know why excesses of ferricyanide should have such relatively little effect upon the standard nor can we explain the discrepancy between our results on the standard thiamine solution and those

[1] Except in the case of germ as will be seen later.

obtained by Conner and Straub (1941) who obtained the same galvanometer readings when a pure thiamine solution was oxidized with amounts of ferricyanide ranging from 0.5 mg to 3.0 mg. Considerable work remains to be done on the excess ferricyanide effect.

When the rapid method is used, duplicate thiamine values can be obtained in approximately 50 minutes, and, under pressure, as many as 64 determinations have been made by a single worker in an 8-hour day. A minor advantage is the fact that much less glassware is required when using this rapid method than when the regular procedure is followed.

Application of rapid method to flours and flour streams. Seventy samples of flour ranging from short patents to low grades were assayed by the rapid method and also by a method which we have referred to as the "regular method." In the "regular method" as already mentioned, oxidation is carried out by adding a mixture of sodium hydroxide and potassium ferricyanide solutions, but in all other essential features it is similar to that outlined in Cereal Laboratory Methods (4th ed. 1941).

TABLE IV

THIAMINE CONTENT OF DIFFERENT GRADES OF FLOUR BY THE RAPID AND REGULAR THIOCHROME METHODS

No samples	Mean ash content	Mean thiamine content		
		Rapid method		Regular method
		As found	Corrected [1]	
	%	μg/g	μg/g	μg/g
4	0.38	0.89	0.94	0.88
21	0.47	1.37	1.46	1.51
11	0.53	1.61	1.72	1.71
11	0.58	2.26	2.42	2.45
7	1.15	6.93	7.46	7.32
16	2.00	13.70	14.78	14.80
Mean		4.89	5.27	5.27

[1] Rapid method corrected by regression equation.

Average results for 70 samples grouped according to ash content are given in Table IV.

There was a correlation of + 0.99 between the results of the two methods and the relationship was that of a straight line which is expressed in the equation:

$Y = -0.02 + 1.08x$ where
Y is the estimated thiamine value for the regular method
x is the thiamine result by the rapid method

The thiamine values obtained by the rapid method were lower than those obtained by the regular method, the magnitude of the difference

being a function of the thiamine content of the flour. Application of the regression equation to the results of the rapid method gave values very close to those obtained by the regular method.

As a further check of the equation, 18 samples of long extraction flour (78–80%) of the type advocated by certain officials of the Canadian government, were tested by both methods. Average results were:

Regular method	2.68 μg/g
Rapid method, as found	2.50 μg/g
Rapid method, corrected	2.68 μg/g

All the flour streams from a commercial mill were tested by both methods and also by the regular method, omitting hydrolysis with enzyme (takadiastase). The results are shown in Table V.

There were very high correlations between the values obtained by the three methods. The rapid method (uncorrected) gave the lowest average value, while differences between the values obtained in the other two series were not significant. According to Booth (1940), wheat contains little or no cocarboxylase and this is borne out, so far as the flour-producing portion of the kernel is concerned, by the fact that omission of digestion with enzyme did not significantly decrease the results obtained by the regular method.

Application of rapid method to wheat. The rapid method was next applied to samples of wheat. The samples were ground so that all except a small amount of branny material passed a 44 GG sieve. Twenty-four samples gave an average value of 4.82 μg/g by the rapid method and 4.70 μg/g by the regular method. Although the difference between the two values is statistically significant, it is too small to warrant the application of a correction factor.

Compared with the regular procedure, the rapid method thus gives low results for flour and essentially correct values for wheat, and one would therefore expect it to give high values on mill feeds. This was found to be the case.

Application of rapid method to mill feeds. The streams going to the feeds in a commercial mill were analyzed for thiamine by the three methods previously used and the results are reported in Table VI.

In the upper section of the table have been grouped the feed flour streams consisting mainly of endosperm, and the feed middlings streams which also contain considerable endosperm. These streams make up but little more than 5% of the total mill products. It will be observed that the average thiamine results obtained by the three methods on these streams display no significant differences.

Shorts and bran together constitute nearly 20% of the total mill products, and contain comparatively little endosperm. These feeds,

as made in Canada, contain respectively 8.0% and 11.5% of crude fiber. For assay by the regular method, with and without enzyme, the bran was air-dried and ground as finely as possible by means of a hand grinder. Both bran and shorts were mixed for a short time with a portion of the extractant in a Waring Blendor before carrying out the

TABLE V

THIAMINE VALUES ON FLOUR STREAMS BY THE RAPID METHOD AND BY THE REGULAR THIOCHROME METHOD WITH AND WITHOUT TAKADIASTASE

Stream	Ash	Thiamine content [1]			
		Rapid method		Regular method	
		As found	Corrected	Standard	Without takadiastase
	%	μg/g	μg/g	μg/g	μg/g
2nd middlings	0.34	0.47	0.49	0.59	0.50
4th middlings	0.37	0.76	0.80	0.80	0.83
1st middlings	0.38	0.47	0.49	0.59	0.53
3rd middlings	0.39	0.76	0.80	0.84	0.77
Coarse sizer	0.41	0.74	0.78	0.78	0.58
Fine sizer	0.43	0.76	0.80	0.85	0.79
5th middlings	0.44	1.09	1.16	1.24	1.18
6th middlings	0.48	1.71	1.83	1.86	1.93
7th middlings	0.48	1.94	2.08	2.10	1.93
2nd and 3rd break	0.51	1.38	1.47	1.68	1.65
1st tailings	0.54	1.53	1.63	1.85	1.84
Roll dust	0.55	1.65	1.76	1.72	1.65
8th middlings	0.64	4.23	4.55	4.24	4.50
4th break	0.68	1.76	1.88	1.74	1.65
2nd quality	0.72	3.70	3.98	4.26	4.13
Purifier dust	0.72	1.82	1.95	2.10	1.74
1st break	0.72	0.82	0.87	0.89	1.01
1st middlings scalps	0.75	3.70	3.98	3.80	3.66
1st chip	0.84	3.03	3.25	3.82	3.68
1st germ	0.93	4.70	5.06	4.45	5.15
1st low grade	0.96	5.29	5.69	6.18	6.19
5th break	1.12	4.17	4.48	4.62	4.54
2nd germ and chip	1.28	8.41	9.06	9.45	8.64
2nd low grade	1.37	9.40	10.13	10.10	9.92
Mean		2.68	2.87	2.94	2.87

Correlation coefficients	r_{xy}
Rapid × regular method	+ 0.996
Rapid × regular (no enzyme) method	+ 0.997
Regular × regular (no enzyme) method	+ 0.996

[1] Single determinations.

extraction in the usual manner. No alteration was made in the rapid method for bran and shorts and in this case unground bran was used. All the bran results were calculated to the same moisture basis. The rapid method gave the highest results and the regular method without enzyme the lowest. These relationships have been confirmed by tests

on other samples. Calculation shows that, taking the data obtained by the regular method as correct, the error in the rapid method values for flour is almost exactly compensated for by opposing errors in the results for the shorts and bran. Because of this, tests by both methods on whole wheat give results which are in close agreement. The lowering of the regular method results on bran and shorts brought

TABLE VI

THIAMINE VALUES ON FEED STREAMS BY THE RAPID METHOD, AND BY THE REGULAR THIOCHROME METHOD WITH AND WITHOUT TAKADIASTASE

Stream	Ash	Thiamine content [1]		
		Rapid method	Regular method	
		As found	Standard	Without takadiastase
	%	μg/g	μg/g	μg/g
(*a*) Low grade tailings	1.78	18.81	18.00	18.94
3rd low grade	2.04	20.77	22.06	20.76
Bran and shorts duster redresser	2.25	14.89	15.07	14.89
Bran and shorts reel	2.30	11.76	13.23	11.76
Feed middlings	2.90	17.28	16.73	15.80
Feed middlings	3.31	18.23	16.54	18.33
Feed middlings	3.75	27.30	28.77	28.29
Mean		18.43	18.63	18.40
(*b*) Shorts	4.19	16.85	15.84	14.85
Bran	5.76	8.00	7.83	7.02
Mean		12.43	11.84	10.94

Correlation coefficient	r_{xy}
Rapid × regular method	+ 0.980
Rapid × regular method (no enzyme)	+ 0.990
Regular × regular method (no enzyme)	+ 0.983

[1] Single determinations, except in the case of bran and shorts which are the means of four determinations.

about by omitting digestion with takadiastase is an indication of the presence of bound thiamine in these by-products.

Wheat germ gave decidedly low results by the rapid method. Two samples of commercial wheat germ were assayed for thiamine by the regular method, with and without takadiastase, and by the rapid method. The means of the results were as follows:

Regular method	23.1 μg/g
Regular method (without takadiastase)	18.5 μg/g
Rapid method (as found)	18.8 μg/g

These results confirm the statement of Andrews and Nordgren (1941) regarding the presence of cocarboxylase in wheat germ and throw doubt upon the values reported by Pearce (1943) who used a simple acid extraction without enzymic digestion in his thiamine assays of germ. Some of the cocarboxylase in bran and shorts must be carried by the germ in these feeds, although they appear to contain more cocarboxylase than can be accounted for in this way. Possibly the bran coat, or some tissue other than germ which is separated with commercial bran and shorts, also contains cocarboxylase.

The rapid method extracts of our germ samples were oxidized with 0.9 mg of ferricyanide. While it was subsequently found that this quantity was insufficient to produce maximum fluorescence, it so happened that the reduction in the fluorescence readings due to the use of *insufficient* ferricyanide was approximately the same as the reduction (about 9%) due to the use of *excess* ferricyanide when 0.9 mg of this reagent was used for the standard etc. as shown in Table II. In the rapid method, germ gives a very high fluorescence blank which rapidly decreases when the isobutanol extract is exposed to ultraviolet light. In our determinations the initial blank reading was used.

TABLE VII

STANDARD ERRORS OF THE RAPID AND REGULAR METHODS

Material	No. samples	Mean thiamine content	Standard error (Single determination)	Coefficient of variability
		μg/g	μg/g	%
Rapid method				
Flour	36	2.45	0.06	2.6
Wheat	18	4.06	0.11	2.7
Bran	11	7.66	0.17	2.2
Shorts	12	17.01	0.57	3.5
Regular method				
Flour	9	0.88	0.05	5.9
Flour	15	1.58	0.06	3.8
Flour	30	2.50	0.10	4.0
Flour	13	3.55	0.12	3.4
Wheat	4	4.67	0.13	2.7
Shorts	9	16.30	0.62	3.8

Precision of the rapid and regular methods. In Table VII are shown the standard errors of the rapid and regular methods, as computed from the results for wheat products of increasing thiamine content.

For both methods the standard error in absolute terms increased with the thiamine content. On a percentage basis (coefficient of variability) however, the error remained fairly constant in the case of the rapid method, and tended to decrease for the regular method as the

thiamine increased. On the average the standard error of the rapid method was slightly lower than that of the regular method.

The thiamine values of several collaborative check samples have been determined by both methods. These included four long extraction flours sent out by the Canadian National Millers' Association and three A.A.C.C. samples of enriched flour. The results, given in Table VIII, show that our data are in reasonably close agreement with the

TABLE VIII

A COMPARISON OF THE THIAMINE VALUES ON CHECK SAMPLES AS REPORTED BY ALL COLLABORATORS AND BY THE AUTHORS

Sample	Values obtained				
	By collaborators		By authors		
	Mean	Range	Rapid (As found)	Rapid (Corrected)	Regular
	μg/g	μg/g	μg/g	μg/g	μg/g
Long extraction flours					
C.N.M.A. No. 4	2.56	2.27–2.88	2.18	2.35	2.37[1]
C.N.M.A. No. 5	2.68	2.39–3.24	2.38	2.57[1]	
C.N.M.A. No. 6	2.69	2.45–2.94	2.49	2.69[1]	
C.N.M.A. No. 7	2.56	2.09–2.89	2.51	2.71	2.82[1]
Mean	2.62		2.39	2.58	
Enriched flours					
Nov. A.A.C.C.	3.89	3.43–5.14	3.59		3.63[1]
Feb. A.A.C.C.	3.81	3.46–4.31	3.69		3.77[1]
Mar. A.A.C.C.	4.03	3.53–4.77	4.08		4.26[1]
Mean	3.91		3.78		3.87

[1] Reported values.

means of the values reported by other laboratories and thus provide evidence as to the general reliability of our figures and the validity of the conclusions reached. Our corrected values for the long extraction flours are in close agreement with the general averages of all laboratories. The uncorrected results of rapid tests on enriched flours are slightly low. Other comparative tests on enriched flours have given similar results. In the rapid method all the added thiamine is extracted and any error is due to the incomplete extraction of the thiamine naturally present. Since this error will usually be very small, no correction is considered to be necessary.

Discussion

The relationship of the rapid method results to those obtained by the regular thiochrome procedure varies with the type of material

analyzed. With wheat and a number of feed streams containing a considerable proportion of endosperm the two methods give results which are in substantial agreement. With germ the rapid method results are nearly 20% lower, on flours they average 8% lower, and on bran and shorts they are higher, than those obtained by the regular method.

The discrepancy in the case of germ can be safely attributed, in a large measure, to the presence of cocarboxylase, but this is not the explanation for the lower rapid method results for flours since they contain little or no cocarboxylase. Some evidence has been presented indicating that the effect of the excess of ferricyanide is more pronounced on rapid method extracts than it is on the standard or on solutions eluted from zeolite. While the use of a fixed ferricyanide level must be recognized as an arbitrary feature and one which may possibly lead to errors in individual cases, our data indicate that it was not the factor responsible for the lower results obtained on flours by the rapid method.

Actually there is, so far as we are aware, no experimental evidence to indicate what is responsible for the low rapid method results on flour, but in any speculation on the subject, the possibility that the thiamine is incompletely extracted from starchy material, under the particular extraction procedure we followed, cannot be altogether overlooked. Nor can we be sure that the foreign material present in the rapid method extract has no effect on the conversion of thiamine to thiochrome or on the partition coefficient between the salt solution and the isobutanol.

The reason for the high rapid method results on bran and shorts is more puzzling since these feeds contain cocarboxylase and would therefore be expected to give low results. Here again the difference does not appear to be related to the excess ferricyanide effects. One of the many possibilities which might be suggested is the presence in bran of soluble substances which, on oxidation, yield products fluorescing in the same range as thiochrome. Another possibility is that our results on bran and shorts by the regular method are low. This would mean, of course, that our results on wheat by both methods are also slightly low.

Whatever the causal factors may be, the discrepancies are relatively small. They average less than 10% in one direction for flour and less than 10% in the other direction for bran and shorts. The results we have reported show that the discrepancy in the case of flour can be removed by the application of a correction factor to the rapid method values and that the rapid method can be applied to wheat and to enriched high grade flour, in which there is a small proportion of natural thiamine, to give results which, without correction, are in close agreement with those obtained by the regular thiochrome method. If

the rapid method is to be applied to other products, the relationship between the regular and rapid method results should be established for each type of material analyzed.

Summary

A modification of the rapid method of Andrews and Nordgren for determining thiamine in wheat and wheat products has been developed for use in flour mill control of the production of enriched and long extraction flours. The method has been standardized against the regular thiochrome method.

With unenriched commercial flours the rapid method gave low results but by the use of a correction factor the values were brought into a close agreement with those obtained by the regular method. With enriched flours and wheat no correction was required. With bran and shorts the rapid method gave higher thiamine values than the regular procedure; with germ it gave lower values.

The presence of cocarboxylase is mainly responsible for the low results with germ, but no explanation has been found for the high results on bran and shorts or for the low results on unenriched flours.

Replicate error was slightly lower for the rapid than for the regular method. For both methods, the absolute errors increased with increasing thiamine content.

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SELECTIVE FERMENTATIONS OF MALTOSE AND LACTOSE IN DOUGHS AS MEASURED BY THE PRESSUREMETER METHOD

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In connection with a study of the utilization of the lactose which is present in the milk products used in baking bread, it was desirable to know to what extent lactose could be made to contribute to the leavening process. A lactose-fermenting yeast was required for this phase of the study, and since it would probably not act upon maltose (but would act upon any dextrose or sucrose present in the flour), the regular "baker's yeast" had to be used in conjunction with it. Baker's yeast ferments dextrose, maltose, and sucrose, but not lactose.

It was also of interest to see in a general way how the results of the test to be adopted would correspond with the diastase value of the flour.

Methods and Materials

Of the several lactose-fermenting yeasts available from the American type culture collection, *Torula cremoris* (No. 2512) was chosen, because the writer's experience indicated that it is the fastest fermenter of this type. Of eight flours available for study, two were chosen: (1) a standard patent flour with a diastatic value of 280 and (2) a short patent flour with a diastatic value of 151. These two flours had the highest and lowest diastatic values, respectively, of the series. Both had a pH value of 6.0.

The gassing values of the doughs were measured in duplicate, by the pressuremeter method recommended by the American Association of Cereal Chemists (*Cereal Laboratory Methods*, 1941), in which a constant quantity of a "straight dough" is enclosed in a vessel and the pressure of the gas evolved in any time interval is read from a mercury column. The writer arbitrarily used 6 hours throughout as the testing period.

Baker's yeast and *T. cremoris* were the fermenting agents. They were used alone, or mixed together in various proportions, but the total amount of yeast was always 0.3 g as required in a standard test. The slight differences in pH of the doughs were ignored.

Experimental

The pressures attributable to maltose were obtained by first carrying out the test in the regular way with baker's yeast as the fermenting

agent. The resulting pressures for both flours are given in Table I, lines A and D. These pressures were obtained from the sugars present and forming during the test, but of course do not indicate total quantities of the different sugars present.

The test was then carried out with *T. cremoris* as the fermenting agent. The results are shown in Table I, lines B and E. This organism does not ferment maltose, so these pressures are considered to be due to dextrose and sucrose, on the assumption that the latter is a natural constituent of flour.

The differences between the values shown in lines A and B, and between those in lines D and E, represent the amount of gas derived

TABLE I

GASSING POWER OF DOUGHS WITH DIFFERENT YEASTS

Line	Yeast	Sugars fermented	Pressures in mm mercury at various hourly intervals					
			1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
			mm	mm	mm	mm	mm	mm
FLOUR NO. 1								
A	Baker's	Sucrose, maltose, dextrose	102	230	378	436	470	500
B	*T. cremoris*	Sucrose, dextrose	30	82	106	114	118	121
C	(A–B)	Maltose	72	148	272	322	352	379
FLOUR NO. 2								
D	Baker's	Sucrose, maltose, dextrose	108	206	276	302	322	338
E	*T. cremoris*	Sucrose, dextrose	38	101	131	143	147	150
F	(D–E)	Maltose	70	105	145	159	175	188

from maltose only (see lines C and F), regardless of whether or not sucrose is present in the flours, since both organisms ferment it. When sucrose is not present the pressures shown in lines B and E are due to dextrose only. The dextrose may be differentiated from the maltose by making use of some of the apiculate yeasts such as the *Klockera* or *Hansenia*,[1] very few of which will ferment a disaccharide. The possibility of any epiphytic microflora in the flours contributing to the data is considered unlikely and is disregarded.

By using these data, pressure values for a part of the flour which the writer has termed the "maltose fraction" (or ratio) can be calculated, since such values apparently indicate the ratio of maltose fer-

[1] A. Klocker: Researches on 17 forms of Sacch. Apiculatus. Compt. rend. (Carlsberg) 10: 285 (1913).

mented to total sugars fermented—that is, only as relative values. This ratio is obtained by dividing C by A for flour No. 1 and F by D for flour No. 2.

The values of this ratio for each time interval for both flours are tabulated in Table II. The relative constancy of these ratios for each flour, after the second hour, seems to offer some justification for the validity of this method of sugar differentiation in the flour.

The fact that the ratios for the two flours differ widely would also appear to offer a method of differentiating between flours themselves, supplementing the diastase test.

TABLE II

"MALTOSE FRACTIONS" DETERMINED AT SUCCESSIVE TIME INTERVALS

Flour	Line	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
No. 1	C/A	0.71	0.64	0.72	0.74	0.75	0.76
No. 2	F/D	0.65	0.51	0.53	0.53	0.54	0.56

In connection with the utilization of lactose for leavening purposes, gassing tests were made as usual but with 6 percent of skim milk powder added (based on weight of flour) and with various portions of the fermenting agent made up of *T. cremoris*. These results are tabulated in Table III. See Table I, lines A and D, for data on baker's yeast with no *T. cremoris*.

TABLE III

GASSING POWER OF LACTOSE CONTAINING DOUGHS WITH DIFFERENT YEAST COMBINATIONS

Line	Yeast fractions		Pressures in mm mercury at various hourly intervals					
	Baker's	T.C.	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
			mm	mm	mm	mm	mm	mm
FLOUR NO. 1								
G	0	1.0	84	189	255	280	293	299
H	0.25	0.75	72	166	274	359	445	527
I	0.50	0.50	90	206	344	480	568	624
J	0.75	0.25	86	204	355	452	504	546
FLOUR NO. 2								
K	0	1.0	70	175	256	293	309	316
L	0.25	0.75	74	174	283	371	447	500
M	0.50	0.50	94	210	334	452	494	520
N	0.75	0.25	93	208	325	367	402	436

If the gas pressures due to maltose (Table I, line C) and those due to lactose, sucrose, and dextrose (Table III, line G) are added together, a measure of the pressure that may be expected in some unknown time would result if the rates of fermentation do not differ greatly. These calculated pressures are shown in Table IV, as C + G and F + K. The actual pressures developed in 6 hours when both yeasts are used together in different proportions, and in the presence of lactose have been tabulated in Table III. In Table III it may be seen that the highest pressures that were developed in the presence of 6 percent of skim milk powder occurred when equal parts of baker's yeast and *T. cremoris* were used as the fermenting agent. (See lines I and M.)

It is of interest to know how closely the actual maximum pressures approach the calculated maximums. This can be expressed as a ratio

TABLE IV

CALCULATED PRESSURES AND ACTUAL FRACTIONS OF THESE PRESSURES DEVELOPED

Pressure[1]	Flour	Pressures in mm mercury					
		1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
		mm	*mm*	*mm*	*mm*	*mm*	*mm*
C + G	No. 1	156	337	527	602	654	678
F + K	No. 2	140	280	401	452	484	504
I/(C + G)	No. 1	.58	.61	.65	.80	.88	.92
M/(F + K)	No. 2	.67	.75	.83	1.00	1.02	1.03

[1] C + G and F + K are calculated pressures; I/(C + G) and M/(F + K) designate ratio of maximum actual pressures to calculated pressures.

by dividing I by C + G for flour No. 1 and M by F + K for flour No. 2. These ratios may be considered to be a measure of the efficacy of the dough fermentations. The results are tabulated in Table IV. The difference between these ratios for the two flours may be attributed in part to the fact that No. 1 flour had the higher diastatic value and consequently maltose was being formed faster than it was being fermented.

Summary

A method of determining the ratio of maltose to total fermentable sugars is described. The technique of this method was used to demonstrate that, by using baker's yeast and *T. cremoris* in equal amounts in a bread dough containing milk solids, all sugars present can be made to contribute effectively to the leavening of the dough.

STARCH FROM EASTER LILY BULBS[1]

NEIL W. STUART[2] and BERNADINE BRIMHALL[3]

(Received for publication September 21, 1943)

In connection with some physiological studies on Easter lilies (Stuart, in press) it was observed that the bulbs contained a high percentage of starch. This starch was easily isolated and appeared to be of such high quality that further investigation of its properties seemed desirable, both from the standpoint of potential industrial uses and for comparison with other native starches in theoretical studies. Reichert (1913) has described the morphology and staining reactions of the starch from two varieties of *Lilium longiflorum*.

The Easter lily, *Lilium longiflorum* Thunb., has been grown in the open for many years in the southern states (chiefly Florida and Louisiana) and in the Pacific northwest. It is an important florist crop for forcing under glass. Prior to 1941 most of the 25,000,000 or more Easter lily bulbs forced annually in this country were imported from Japan. At the present time domestic production of Easter lily bulbs is being successfully expanded.

Easter lilies are propagated from seeds, stem bulblets, and bulb scales. Since the seedlings vary in many characters, multiplication of stocks that are to be genetically identical must be by asexual means. The Easter lily plant forms offsets from the main bulb, and smaller bulblets on the underground portion of the stem. These bulbs, graded into various sizes, are used for planting stock. Lilies may also be propagated from bulb scales. The so-called scales are overlapping fleshy segments of the main bulb. When removed from the bulb these scales will form several bulblets that will in a year's time equal or surpass stem bulblets in size.

Easter lily bulbs are planted in the fall and are harvested about 10 months later. The yield of bulbs will vary, depending upon the size of planting stock, soil and climatic conditions, and incidence of disease. Mixed seedling Easter lilies grown in rows 3 feet apart at Beltsville, Maryland, have yielded at the rate of 9,000 pounds of bulbs per acre. With more intensive cultural practices this yield has been more than doubled. For example, a bed 116 feet long and 5 feet wide planted with bulb scales yielded 585 lb of bulbs. Additional information concerning the production of Easter lily bulbs is given by Emsweller and Brierley (1942).

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[3] Research Associate, Plant Chemistry Subsection, Iowa Agricultural Experiment Station, Ames, Iowa.

Since Easter lilies are forced throughout the year, it is necessary to hold the bulbs in cold storage for varying lengths of time. Studies in progress at the Bureau of Plant Industry Station show that cool storage of the bulbs results in prompt hydrolysis of a portion of the starch to sucrose and reducing sugars. A storage period of one week at a temperature of 10°C increased the total sugar content of the bulb scales from 2.8% to 7.2% of the total solids. The starch used in the present study was obtained within a month after the bulbs were harvested and prior to any cold storage treatment.

Preparation of the Starch

The milling of the bulbs was carried out in a small scale laboratory unit (Hixon and Sprague, 1942) by a procedure similar to that used

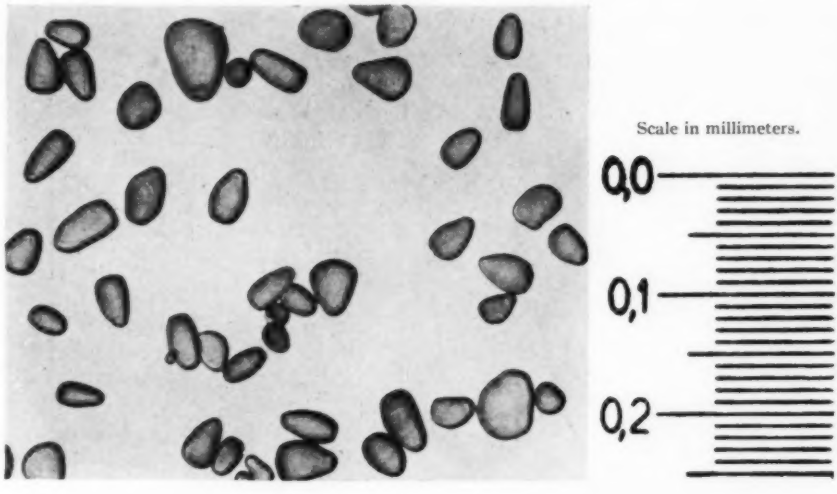

Fig. 1. Lily bulb starch, weakly stained with iodine.

commercially for milling potatoes (Radley, 1940). The bulbs, from which the roots had been removed, were pulled apart and passed with water through a small Buhr mill until the greater portion of the starch could be freed from the accompanying fiber by sieving and screening. The total fiber, along with some starch which was retained in it, amounted to about 2% by weight of the original bulbs. After running through jigger screens, the starch suspension was passed into a basket centrifuge revolving at a speed such that most of the proteinaceous impurities stayed in the overflow water. The centrifuged solids were mixed with fresh water and, upon settling, the remaining protein formed a clear-cut, dark layer on top of the starch from which it could

be washed off. Finally the starch was filtered on a Buchner funnel and the resulting white cake dried in a stream of warm air at 40°C. In three separate milling runs starting with 20, 50, and 60 lb of bulbs, there were obtained 2.5, 5, and 6 lb of starch, respectively, a yield of 10% to 12% based on the green weight of bulbs. The starch had a nitrogen content of 0.028%. When the starch was hydrolyzed with

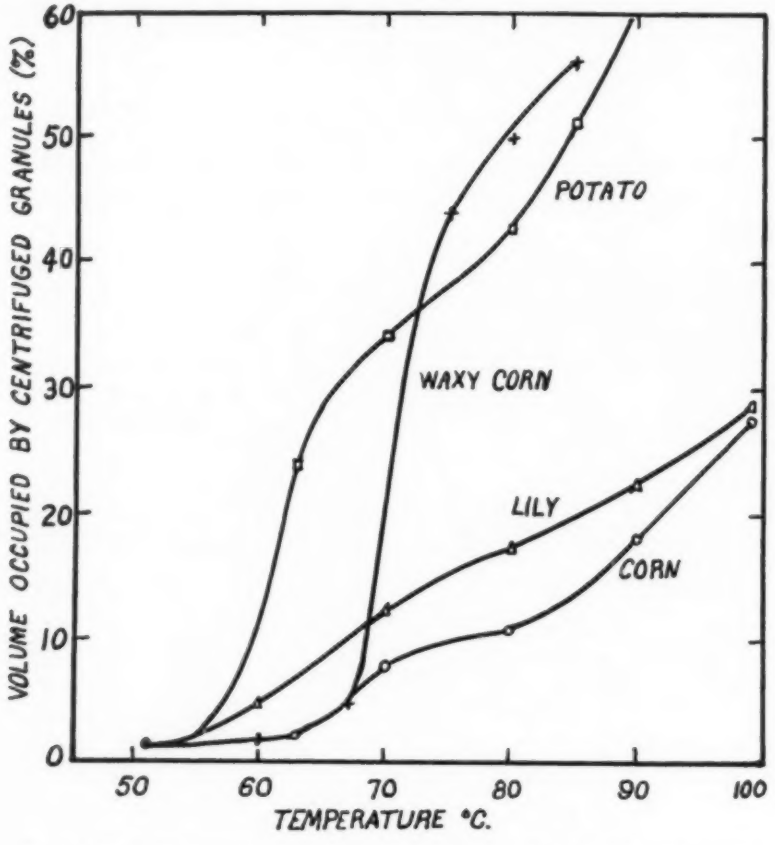

Fig. 2. Effect of temperature on swelling of four starches as shown by volume of granules.

boiling 5*N* hydrochloric acid and the insoluble residue ignited, 0.17% ash remained. This was made up chiefly of silica.

Properties of the Starch

As may be seen from Figure 1, the granules are not uniform in size, ranging from about 20 × 20 microns to 50 × 70 microns, the average being 25 × 45 microns. The color with iodine is a clearer, purer blue than that given by corn or potato starches. The larger granules begin

to swell at 54° to 55°C. At 60°, slightly over half of them are swelling, but a temperature of about 64° is required before all the smaller granules swell as observed microscopically. The unswollen starch has an X-ray pattern closely resembling that of potato starch.

The curves in Figure 2 show the relative increase in volume of the granules as temperature increases for lily, potato, corn, and waxy corn starches. The data were obtained by suspending one gram of starch in 100 ml of water, heating 30 minutes at the desired temperature, cooling, and centrifuging in graduated tubes.

$$\text{Volume of granules } (\%) = \frac{\text{Volume of centrifuged granules} \times 100}{\text{Total volume of suspension}}.$$

Lily starch resembles corn starch rather than tapioca or potato in the opaque appearance and "toughness" of its pastes and the manner in which their consistency varies with temperature. Viscosities were determined on 3% pastes at 85° and 90°C by the capillary method (Brimhall and Hixon, 1942) under 13 cm pressure. The higher temperature results in a lower viscosity for the tapioca paste:

Starch	Viscosity (Centipoises) 85° C	90° C
Tapioca	88	62
Lily	16	22
Corn	2	7

Lily starch has a higher rigidity (Brimhall and Hixon, 1939) or gelling tendency than does corn starch:

Temperature of preparing the paste	Rigidity at 25° C (dynes per sq. cm. $\times 10^{-1}$) 4.5% pastes	
	Lily	Corn
85° C	41	6
90	192	96
95	264	144
99	280	150

In contrast, the rigidity of potato and tapioca starches reaches a maximum at about 70°C and decreases rapidly as temperature of preparing the paste increases. The pastes of lily starch are claimed by some observers to have an odor resembling that of the bulbs themselves, but this can probably be removed by proper treatment.

Using the potentiometric iodine titration method (Bates, French, and Rundle, 1943) lily starch was found to have the largest proportion of unbranched molecules (30 to 34%) of any native starch so far ex-

amined, a factor probably responsible for the deeper blue iodine color mentioned above. Potato starch contains only 21% of this component and defatted cornstarch, about 27% (Schoch, 1943).

The fractionation of lily starch by the butanol method (Schoch, 1942) proceeds smoothly and preliminary defatting of the sample is unnecessary since less than 0.1% of fatty acids is present. The butanol-nonprecipitated fraction, consisting chiefly of branched molecules (amylopectin), could be 62% converted to maltose by soybean β-amylase as compared to 55 to 58% conversion of tapioca, potato, and corn starch amylopectins under identical experimental conditions. When a 1 or 2% solution of lily amylopectin was frozen and then allowed to thaw at room temperature, it assumed the spongy, fibrous texture characteristic of retrograded starch, a phenomenon not exhibited by potato or tapioca amylopectins. These results might be explained by assuming longer branches on the lily amylopectin molecules.

As the knowledge of various starch types is extended, it becomes apparent that the terms "cereal" and "tuber" are significant only in indicating the source; they allow no inferences as to the properties of the starch.

Summary

At the present time domestic production of bulbs of the Easter lily, *Lilium longiflorum* Thunb., is being successfully expanded. A high quality starch is easily isolated from the bulbs. It resembles potato starch in granule size, gelatinization temperature, and X-ray pattern, but the properties of its pastes—appearance, consistency, and variation with temperature—are more nearly like those of corn starch.

Acknowledgment

These studies were made in the Plant Chemistry Subsection of the Iowa Agricultural Experiment Station, using starch isolated from bulbs grown at the Bureau of Plant Industry Station, Beltsville, Maryland. Mr. Welker Bechtel took the photomicrograph of the starch granules.

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THE EFFECT OF DIFFERENT OPERATORS ON THE EVALUATION OF MIXOGRAMS [1]

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(Received for publication September 12, 1943)

The value of the mixograph as a supplementary device in the testing of wheat varieties for quality is becoming generally recognized. Quantitative mixogram measurements recently made at the North Dakota Agricultural Experiment Station showed very significant differences in dough development stage and range of stability between hard red spring wheat varieties and also between the stations at which the varieties were grown. Johnson, Swanson, and Bayfield (1943) also discussed relations between mixogram measurements and their utility in the estimation of wheat quality. No tests apparently have been made of the possibility of obtaining reasonable agreement between curves secured by different operators.

In this laboratory it has been found convenient to discuss mixograms in terms of measurements of certain of their dimensions. In the past it has been the practice to have all measurements for any one investigation made by the same operator, but this is not a convenient procedure in large investigations. Accordingly, it seemed advisable to undertake a study of the agreement between curve measurements made by independent operators. The results are reported in this paper.

Procedure

Ten samples of straight grade flour of approximately 70% extraction experimentally milled from hard red spring wheat varieties grown in comparable plots in 1942 were used in this experiment.

[1] Published with the approval of the Director of the Experiment Station.

The wheats from which the flours were milled were sound. The flours were diluted with experimentally prepared wheat starch to a common protein level of 12.0% (13.5% moisture basis). The mixogram curves were made with 25 g of flour (13.5% moisture basis) and the required quantity of distilled water to correspond to the absorption used in baking. The mixer was operated from 8 to 10 minutes, depending upon the variety under test.

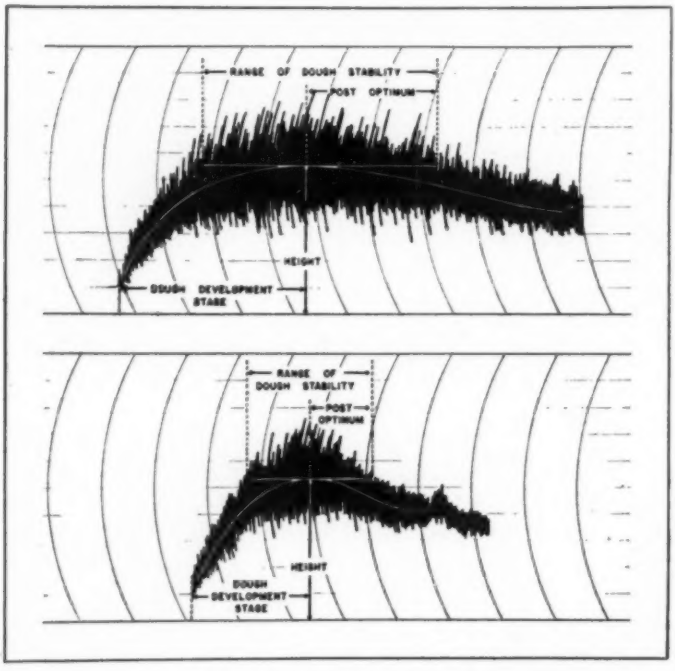

Fig. 1. Representation of the method used in securing the mixogram property measurements. Upper curve was made from a flour with long mixing requirements and range of dough stability. Lower curve was from a flour having an exceptionally short dough development stage and poor dough stability.

Figure 1 shows the method followed in measuring the mixograms. A point is located at the center of the curve and at its greatest height. A vertical line is dropped through this point to the base line; and another line is drawn through the point parallel to the base. The measurements taken from the mixogram (in cm), and the names given them, are self evident from the figure.

The measurements were carried out in duplicate by three persons. Two of the operators were experienced in judging hard red spring wheat mixer curves, while the third was without previous experience but had been given sufficient instruction to become familiar with the evaluation of curve properties. Each operator carried out his share of the work

independently, including the mixing and estimation and measurement of curve dimensions. The samples were designated by number and the absorptions used were those determined when baking the flours. No comparisons of results were permitted and no instruction was given to any worker during the course of the work.

Differences in curve properties due to variations in protein content were eliminated by using flours at a uniform protein content, as is the

TABLE I

AVERAGE ESTIMATION OF DOUGH DEVELOPMENT, DOUGH STABILITY, AND CURVE HEIGHT, BY THREE OPERATORS

Property	Sample No.	Operators			Mean
		A	B	C	
		cm	*cm*	*cm*	*cm*
Dough development	1	10.5	11.8	12.2	11.5
	2	10.2	11.4	12.4	11.3
	3	8.2	8.7	9.2	8.7
	4	5.4	5.8	6.8	6.0
	5	5.6	5.6	6.2	5.8
	6	5.1	5.8	6.1	5.7
	7	5.2	5.4	5.7	5.4
	8	5.1	5.2	5.2	5.2
	9	4.4	4.2	4.2	4.3
	10	2.6	3.4	3.9	3.3
Mean		6.2	6.7	7.2	6.7
Standard deviation		2.315	2.785	2.919	
Dough stability	1	6.0	5.4	8.2	6.5
	2	6.4	5.6	6.8	6.3
	3	6.3	5.5	3.9	5.2
	4	4.0	5.0	5.0	4.7
	7	3.4	5.0	4.6	4.3
	5	4.6	4.6	3.6	4.3
	8	4.0	4.3	4.5	4.3
	9	3.0	3.3	3.9	3.4
	6	3.0	3.8	3.0	3.3
	10	3.0	3.4	3.4	3.3
Mean		4.4	4.6	4.7	4.6
Standard deviation		1.446	0.887	1.576	
Curve height	5	7.8	7.6	7.4	7.6
	3	7.7	7.8	7.2	7.6
	9	7.6	7.6	7.4	7.5
	10	8.1	7.0	7.0	7.4
	1	7.8	7.2	7.0	7.3
	6	8.1	6.9	6.8	7.3
	7	7.2	7.2	6.7	7.0
	4	7.8	6.9	6.3	7.0
	8	7.4	6.8	6.8	7.0
	2	7.4	6.6	6.2	6.7
Mean		7.7	7.2	6.9	7.3
Standard deviation		0.327	0.413	0.406	

usual practice in this laboratory. Duplicate measurements provided an estimate of the experimental error for each operator, while the use of three operators decidedly strengthened the conclusions derived from the comparisons.

Results and Discussion

In Table I are shown the average results obtained by the three operators on the 10 flours with means and standard deviations. The duplicate readings of each operator were in much better agreement than the results between operators. The samples are arranged in the top section in decreasing order of dough development, while in the second and third sections decreasing orders in respect to stability and curve height have been followed. This arrangement renders it possible to see very easily whether any correlation exists between the three curve properties. A positive relation is apparent between dough development and dough stability, as found by Harris, Sibbitt, and Elledge (1943). The means increase in value from A to C for dough development and stability, but decrease in the same order for curve height. Operator C tended to have more variability in results for dough development and stability.

An analysis of the data was made to determine the significance of the differences between samples and operators. The results are shown in Table II. The significance of the variances due to samples

TABLE II

ANALYSES OF VARIANCE

Cause of variance	Degrees of freedom	Variances		
		Dough development	Dough stability	Curve height
Samples	9	47.00††	7.97††	0.55
Operators	2	3.75††	0.46	3.38**
Samples × operators	18	0.33**	1.26**	0.15
Duplicates	30	0.13	0.43	0.40

†† Significantly greater than the mean square due to samples × operators.
** Significantly greater than the mean square due to duplicates.
Double signs denote that the significance exceeds the 1% level.

and operators was found by comparison with the variance for the interaction between samples × operators, while the latter variance was tested for significance by comparison with the duplicate variance. It is apparent that the differences in dough development stage and range of stability among samples was much greater than among operators; in fact for stability the operator differences were non-significant. In curve height, the operators failed to obtain significant

differentiation among samples, but differed significantly among themselves. There is little doubt that these results would have been reversed if flours at the original protein content had been used, as the uniform protein level tended to obviate differences in curve height. There were very significant interactions between samples and operators for dough development and stability, but none for curve height. That is, the individual operators did not estimate dough development and stability in exactly the same manner for the different flours.

Correlation coefficients between the data obtained by the three operators are shown in Table III. It is evident that little difference

TABLE III

CORRELATION COEFFICIENTS FOR OPERATOR COMPARISON DATA
(Value of r_{xy} at 5% point = 0.482)

Measurement	Operators	Correlation coefficient
Dough development stage	A × B	+ 0.985
	A × C	+ 0.979
	B × C	+ 0.990
Dough stability	A × B	+ 0.644
	A × C	+ 0.590
	B × C	+ 0.605
Curve height	A × B	+ 0.183
	A × C	+ 0.232
	B × C	+ 0.757

exists among operators in their ability to estimate dough development stage. The correlations for range of dough stability are rather low, though they are significant, and it is evident that the readings obtained by one operator will not allow for the prediction of the values secured by another worker with great accuracy. For curve height the only significant coefficient is that between operators B and C.

In making comparisons between different mixographs it would be necessary to standardize the machines very carefully before undertaking comparative varietal evaluations. There is little doubt that differences between mixers might very easily introduce much larger errors than would be due to the variability among operators. It would have been interesting to investigate this point following the conclusion of the work reported, but no additional equipment was available for making such a study. Operators without extensive experience can apparently successfully evaluate mixogram properties by the method employed in this experiment. The results confirm the general opinion of cereal technologists that distinct varietal differences in these properties exist among wheat varieties.

Summary

A comparative study was made of the estimation of dough development, dough stability, and curve height of mixograms by three operators employing 10 hard red spring wheat flours adjusted to a uniform protein level of 12.0% by dilution with wheat starch. The mixogram measurements were made independently in duplicate by each operator.

Significant differences between operators were found for dough development and curve height, but not for dough stability. There were significant interactions between varieties and operators for dough development and stability, but not for curve height.

The results obtained by different pairs of operators for dough development were highly correlated. The corresponding correlations were lower for dough stability, while no significant correlation was found in two of the comparisons between operators and curve height.

Differences in curve properties among samples were very much greater than operator effects, except for curve height. If flours at the original protein content had been used there would, no doubt, have been significant differences for curve height among the samples.

Literature Cited

Johnson, J. A., Swanson, C. O., and Bayfield, E. G.
1943 The correlations of mixograms with baking results. Cereal Chem. **20**: 625–644.

BOOK REVIEWS

The Chemistry of Large Molecules (Frontiers in Chemistry, Vol. I.) Edited by R. E. Burk and Oliver Grummitt. 313 pages. Interscience Publishers, Inc., New York, N. Y. 1943. Price $3.50.

The following quotations from the preface will make clear the admirable plan of the *Frontiers in Chemistry*. "The burdens of keeping abreast with the forward movement of science must be felt even by the brightest minds and the most energetic souls in that profession. This problem presents itself with particular force to Universities who seek to train graduate students to be well-informed research workers." . . . "The problem of keeping abreast of science is also an important one to industrial research departments and to the individuals therein. There is a great tendency for the individual industrial research man to work in a rather narrow practical field." . . . "To meet these difficulties, Western Reserve University conceived the idea of inviting distinguished scientists in the Field of chemistry and closely related fields to be professors for a day. Each of these men presents two lectures. This gives him an opportunity to present a substantial view of his work. The lecturers who follow present somewhat different, though definitely related fields, so that at the end of a series one has what is, after all, a connected course of lectures, but one in which each lecture is given by a real expert."

Volume I is devoted to The Chemistry of Large Molecules. There are eight chapters dealing with the following topics:

(1) The Mechanism of Polyreactions. By H. Mark, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.
(2) The Investigation of High Polymers with X-Rays. By H. Mark.

(3) The Colloidal Behavior of Organic Macromolecular Materials. By Elmer O. Kraemer, Biochemical Research Foundation of the Franklin Institute, Newark, Del.
(4) The Ultracentrifuge and its Application to the Study of Organic Macromolecules. By Elmer O. Kraemer.
(5) Elastic-Viscous Properties of Matter. By Arthur Tobolsky, Richard E. Powell, and Henry Eyring, Princeton University, Princeton, N. J.
(6) The Electrical Properties of High Polymers. By Raymond M. Fuoss, General Electric Co., Schenectady, N. Y.
(7) Organic Chemistry of Vinyl Polymers. By C. S. Marvel, University of Illinois, Urbana, Ill.
(8) Chemistry of Cellulose and Cellulose Derivatives. By Emil Ott, Hercules Powder Co., Wilmington, Del.

These titles are sufficiently descriptive to indicate the content of these chapters and the list of collaborators in this undertaking shows that the editors have been eminently successful in achieving their aim of having each lecture given by a real expert. Volume I of *Frontiers in Chemistry* sets a high standard, and the collaborators, editors, and publishers are to be congratulated.

WALTER M. LAUER,
University of Minnesota,
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The Microscope and its Use. By Frank J. Muñoz and Harry A. Charipper. xii and 334 pp. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1943. Price $2.50.

Here is a brief book on the construction and use of the microscope which covers a number of specialties as well as the principles. The chapters include the Evolution of the Microscope, the Modern Microscope, Illumination, the Microtome, Use and Care of the Microscope, the Stereoscopic Microscope, the Metallurgical Microscope, the Polarizing Microscope, Accessories for Use with the Microscope, and Common Errors in the Use of the Microscope. It apparently is a translation and an expansion of an earlier book by one of the authors—*El Microscopio y su Uso.*

Unfortunately not all the qualities indicated in the preface (p. iii) as desirable for "such a guide" are satisfactorily achieved. In spite of the statement that "such a guide should be written . . . in very clear, non-technical language . . ." the book abounds in awkward constructions such as, "To get real critical illumination . . . we would have to focus with our condenser our light source on the object at the same time that the object is in focus" (p. 63); and orphan expressions posing as sentences, "No hesitation once the section has been begun" (p. 104); or, ". . . illumination follows a square law in relation to distance" (p. 277). Colloquialisms flavor the book, for instance, ". . . it is O.K." (p. 109); or ". . . when the 'match' is made, it is sometimes blown up some when making the photomicrograph . . ." (p. 197). It can be agreed, however, that it is "non-technical."

The book leaves one with the impression that much of the material is to be found in the trade literature, although occasional shrewd and helpful comments may be ferreted out. Free use has been made of cuts provided by the various manufacturers of microscopes and accessories.

An exception to the curious style pervading the book is to be found in the 40 pages devoted to the polarizing microscope. Mr. William F. Butler, according to the Acknowledgment (p. viii), "contributed greatly to the content and structure" of this chapter. Within the limited space available there has been presented an excellent elementary treatment. For special applications and details of theory, however, the user of the polarizing microscope will continue to refer to the standard works.

The food analyst will find little detailed information of use, although if he cares to encounter the turgid style of the book he may discover an occasional useful hint concerning the practice of microscopy.

The book would have been useful to the beginner in microscopy, if it had been carefully organized and if it had been written in acceptable English.

E. C. ABBE,
University of Minnesota,
Minneapolis, Minn.

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AUTHORS

SUBJECTS

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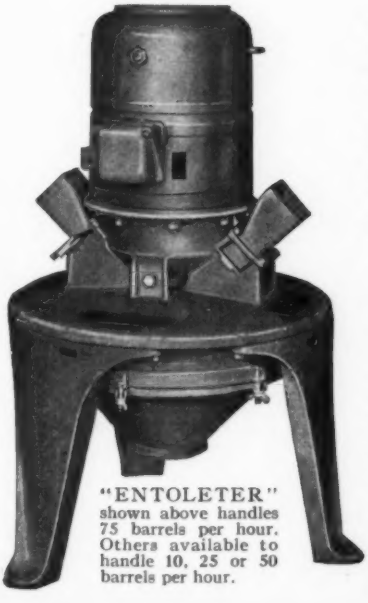

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